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Patentanmeldung Nr. Patent application No. Demande de brevet n°

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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se référer à la description.)

Pharmaceutical composition

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Pharmaceutical composition***Field of the invention***

5 This invention relates to pharmaceutical compositions and methods for treating neoplasms, in one preferred embodiment to neoplasms of the brain such as glioma or astrocytoma.

Antineoplastic chemotherapeutic agents and radiation are the most common agents and methods, besides surgery for the treatment of neoplasms. Antineoplastic chemotherapeutic agents comprise e.g. alkylating agents, anti-metabolites and alkaloids derived from plants. The common effect of these
10 antineoplastic chemotherapeutic agents and radiation is the unspecific inhibition of the cell proliferation and the unspecific induction of cell death respectively, by a wide range of different mechanisms not completely discovered so far.

The inhibition of cell proliferation and the induction of cell death, respectively,
15 primarily influences rapidly growing cells such as tumor cells. But at the same time the proliferation of other rapidly growing cells such as cells of the hair follicle, colon mucosa cells and also immune cells is inhibited. The immune cells inhibited are for example T-lymphocytes, B-lymphocytes, granulocytes, macrophages, microglia cells as well as the respective precursor cells of the
20 bone marrow. The administration of antineoplastic chemotherapeutic agents unspecifically inhibiting cell growth therefore is associated with severe side effects and general suppression of the function of the immune system, which is proven by a lot of "in vitro" and "in vivo" results. For example, dacarbazine which cannot be clearly classified according to standard classification so far is
25 reported to inhibit the humoral and cell-mediated immune response in mouse cells (Giampietri 1978, Nardelli 1984). The same results can be found for temozolomide which is an active metabolite of dacarbazine. Further "in vitro" studies of temozolomide show inhibition of cytotoxicity of lymphocyte activated killer cells (Alvino et al. 1999). CCNU (lomustine) as a representative for alky-

lating antineoplastic agents was shown to suppress both T-cells and B-cells (Bernego et al. 1984) by e.g. suppressing T-cell mediated cytotoxicity and further suppresses T-cell mediated cytotoxicity. (Einstein et al. 1975). Further "in vitro" comparative studies of the alkylating agent cyclophosphamide, the
5 antimetabolite 5-fluorouracil, the alkaloid vincristin and the antibiotic doxorubicine commonly clearly show suppression of the cytotoxic T-cell function (Gereis et al. 1987).

Whereas the chemotherapy of some neoplasms is very successful, many neoplasms are accompanied by a poor life expectancy.

- 10 Another approach in the therapy of neoplasms is the stimulation of the immune system. There is a wide range of stimulators of the function of the immune system and/or the immune cells e.g. immune cell attracting substances, viruses and molecules involved in antigen processing, presentation or trans-
15 porting, fusion cells of dendritic and tumor cells. Antagonists of substances downregulating the function of the immune system are regarded as stimulators of the immune system as well.

As a common principle these immune stimulators employ the ability of the immune system to selectively kill "foreign" tumor cells while sparing other fast growing "self" cells. This is of course an superior approach for treating neo-
20 plasms compared to unspecific inhibition of all growing cells or unspecific destruction of cells of an organism, respectively, which is the principle of the above mentioned antineoplastic chemotherapeutic agents as well as of radiation.

One example for a potent inhibitor of the immune system is TGF-beta (transforming growth factor-beta) mediating the neoplasms' escape from
25 immunosurveillance (Wojtowicz-Praga, S., 1997). Cellular immunity is highly suppressed in patients suffering from neoplasms producing high levels of TGF-beta (de Visser, K.E. et al. 1999).

Using a substance specifically inhibiting the TGF- β production and thus stimulating the immune system is a promising approach for the treatment of neoplasms (Stauder, G. et al. 2003).

5 Despite these promising results the tumor therapy with immunostimulators seems to have margins at least in very quick-growing tumors.

Therefore there is an urgent need for the development of new therapeutics also for the treatment of fast growing neoplasms that are more reliable, have less side effects and increase the life spans of patients suffering from neoplasms.

10 In a clinical phase II study, upon administration of an immunostimulatory agent (antagonist of the immunosuppressor TGF- β), we surprisingly recognized that the median overall survival of patients treated with an antineoplastic chemotherapeutic agent before the treatment with this immunostimulant agent was clearly longer compared to patients not treated with an antineoplastic chemotherapeutic agent:
15

Since the antineoplastic agents suppress the immune system by inhibiting the proliferation of the immunocompetent cells, as described above, up to now the approach of combining these antineoplastic agents with stimulators of the immune system in human beings were deemed not to be an appropriate approach for tumor therapy or tumor medicaments. In the literature reporting
20 about neoplasm therapy by stimulation of the immune system it is emphasized that there has to be a sufficient time delay between the administration of a chemotherapeutic agent and a substance stimulating an immune response (e.g. Timmermann, J. M. 2002).

25 ***Summary of the invention***

It was found that patients treated with a combination of an antineoplastic chemotherapeutic agent and an stimulator of the immune system surprisingly showed significant longer life spans compared to patients treated with either of

these therapeutics.

The invention comprises a pharmaceutical composition with a stimulator of the function of the immune system and/or immune cells and substances inhibiting cell-proliferation and/or inducing cell-death.

- 5 In a preferred embodiment the invention is a pharmaceutical composition comprising at least one antagonist of TGF-beta and at least one antineoplastic chemotherapeutic agent. The at least two substances are mixed or are separate.

- 10 In yet a more preferred embodiment the antagonist is selected from the group of TGF-beta specific nucleotides, TGF-beta binding proteins, TGF-beta binding receptors, parts TGF-beta binding receptors, TGF-beta specific peptides and low molecular substances binding TGF-beta or any other protein, receptor, part of receptor protein or low molecular substance inhibiting the function of TGF-beta.

- 15 In yet a more preferred embodiment the TGF-beta specific nucleotides are antisense oligonucleotides of TGF-beta.

- 20 Methodes to treat neoplasms are also part of this invention. The substances or methods stimulating the function of the immune system and/or the immune cells are administered with substances inhibiting cell proliferation and/or inducing cell death. The substances are administered by any known route in the art for administering medicaments.

The at least two substances of the pharmaceutical compositions according to this invention are mixed together or separately, optionally in the same carrier formulation or in separate pharmaceutical carriers.

- 25 The treatment of a patient suffering from unwanted neoplasms with a pharmaceutical composition as described above, in a preferred embodiment additionally with radiation is also part of this invention.

The at least two substances of the pharmaceutical compositions according to this invention are administered parallel, before or after each other, by the same route or by different routes, together with the radiation, before or after the radiation.

5 Advantages

Patients suffering from neoplasms that were treated with at least one substance stimulating the immune system and/or the immune cells together with a substance inhibiting the cell proliferation and/or inducing cell death show, and/or radiation show clearly longer life spans compared to patients treated
10 with each of these medicaments alone.

This can lead to a reduction of the dosage of one of these medicaments being administered and thus to the reduction of potential undesirable side effects.

Figures

Figure 1 depicts a comparative study of lymphokine activated killer cell (LAK
15 cell) mediated cytotoxicity on glioma cells. One part of the cells was incubated with the TGF-beta 2 specific antisense oligonucleotide with Seq. Id. No. 30 the other part was additionally treated with CCNU. The figure clearly points out that the cytotoxic activity of LAK cells treatment with CCNU in combination the TGF-beta 2 specific antisense oligonucleotide with Seq. Id. No. 30 is superior
20 compared to LAK cells treated with only TGF-beta 2-specific antisense oligonucleotide with Seq. Id. No. 30.

5x10⁶ PBMC (peripheral blood mononuclear cells) were cultivated in 4 µL RPMI 1640 medium supplemented with 10% foetal calf serum, in the presence of 10 ng/ml rh IL-2 (recombinant human interleukine 2), in 5% CO₂ atmosphere at
25 37°C. The first 3 days 5 µM TGF-beta 2 specific antisense oligonucleotide with Seq. Id. No. 30 was added. After that one part of the cells was incubated with 10 µM CCNU for additional 6 h. Cell-mediated cytotoxicity, quantified by CARE-LASS assay (Lichtenfels et al., 1994), of LAK cells treated with TGF-beta 2

specific antisense oligonucleotide with Seq. Id. No. 30 (horizontal hatchures) was compared to LAK cells treated with TGF-beta 2 specific antisense oligonucleotide with Seq. Id. No. 30 in combination with CCNU (diagonal hatchures). Indicated are means \pm SD of quadruplicates.

5 **Figure 2** depicts a comparative study of lymphokine activated killer cell (LAK cell) mediated cytotoxicity on glioma cells. One part of the cells was incubated with the TGF-beta 2 specific antisense oligonucleotide with Seq. Id. No. 30 the other part was subsequently treated with Temozolomid (TMZ). The figure clearly points out that the cytotoxic activity of LAK cells treatment with temo-
10 zolomid after the treatment with TGF-beta 2 specific antisense oligonucleotide with Seq. Id. No. 30 is superior compared to LAK cells treated only with TGF-beta 2 specific antisense oligonucleotide with Seq. Id. No. 30. 5×10^6 LAK were cultivated in RPMI medium supplemented with 10% foetal calf serum, in the presence of 10 ng/ml rh IL-2 (recombinant human interleukine 2), in 5% CO₂
15 atmosphere at 37°C. The first 3 days 5 μ M TGF-beta 2 specific antisense oligonucleotide with Se CARE-LASS assay (Lichtenfels et al., 1994) in one part of the cells without further treatment (only TGF-beta 2 specific antisense oligonucleotide with Seq. Id. No. 30, horizontal hatchures), in the other part in the presence of 30 μ M Temozolomid (TMZ, diagonal hatchures). Indicated are
20 means \pm SD of quadruplicates.

Seq. Id. No. 30 was added. Cell-mediated cytotoxicity was then quantified by
25 **Figure 3** depicts survival data of patients treated with the TGF-beta antisense oligonucleotide with Seq. Id. No. 30 after treatment with Temozolomid according to standard schedule compared to the median overall survival time of patients treated with only Temozolomide according to standard schedule. Survival time is given from start of first chemotherapy after tumor recurrence. Median overall survival time in the clinical study is evaluated from 3 patients with Anaplastic Astrocytoma and 10 patients with glioblastoma. The survival data are compared to the survival data of the literature. Our data reveal longer
30 median overall survival times if applying TGF-beta 2 specific antisense oligonucleotide with Seq. Id. No. 30 following temozolomide than the comparable

published data for temozolomide alone: 146.6 weeks vs. 42.0 weeks for patients suffering from Anaplastic Astrocytoma and 45.1 weeks versus 32.0 weeks for patients suffering from glioblastoma.

Detailed description of the invention

General

References of literature, patents or publications of patent applications mentioned in the description are incorporated by reference.

The method of the present invention is applicable to any mammal. Examples of mammaly to which the method may be usefully applied include laboratory animals, including rodents such as mice, rats and guinea pigs; farm animals such as cows, sheep, pigs and goats; pet animals such as dogs and cats; and primates such as monkeys, apes and humans. The invention is most preferably applied in human clinical situations, particularly where the patient is undergoing immunosuppressive therapy after organ or tissue transplantation, or any other form of surgery where suppression of the immune system of the patient is indicated. However, other mammaly may also benefit from the practice of the invention. These other high value animals such as horses and fur animals such as mink.

Kit of parts

In one embodiment of this invention the at least one stimulator of the function of the immune cells and/or the immune system and at least one substance inhibiting the cell proliferation and/or inducing cell death is a mixture of these at least two components pure or in a pharmaceutical acceptable carrier also herein referred to as combination.

In another embodiment of this invention the at least one stimulator of the function of the immune cells and/or the immune system and at least one substance inhibiting the cell proliferation and/or inducing cell death are separate in one pharmaceutical composition. Each of these parts being pure or in a phar-

maceutical acceptable carrier. The at least two parts of the pharmaceutical composition have the same or different pharmaceutical acceptable carriers. To these separate parts of a pharmaceutical composition is also referred to herein as combination.

5 Immune cells

Immune cells are lymphoid cells, such as T cells, B cells, NK cells (natural killer cells), NK T cells (natural killer T cells), granulocytes, such as neutrophils, eosinophils, basophils, and mononuclear cells such as monocytes, macrophages, dendritic cells and mast cells.

10 Immunostimulator

An immunostimulator according to this invention is any substances inducing the the function of immune cells and/or the immune system to enhanced abilities directly or indirectly reducing or inhibiting the tumor cell growth and/or inducing cell death of unwanted neoplasms in a pharmaceutical acceptable carrier.

15 Apparatus and/or methods inducing the function of the immune cells and/or the immune system as described above are also within the scope of this invention.

In one embodiment the immunostimulator is selected from the group of chemokines, including but not limited to lymphotactin, interleukine 1, interleukine 2, interleukine 6, interleukine 12, interferon gamma, and/or immune
20 cell attracting substances.

In yet another embodiment the immunostimulator is selcted from the group of viruses and/or parts of viruses, including retroviruses, adenoviruses, papillomaviruses, Epstein-Barr-viruses and viruses that are non-pathogenic including Newcastle-Disease virus, Cow-pox-virus

25 In another embodiment the immunostimulator is selected from the group of autologous, heterologous MHC-Molecules, molecules involved in antigen processing, molecules involved in antigen presentation, molecules involved in me-

diating immune cell effects, molecules involved in mediating immune cell cytotoxic effects, molecules involved in antigen transportation, co-stimulatory molecules, peptides enhancing recognition by immune cells and/or cytotoxic effects of immune cells.

- 5 In yet another embodiment the immunostimulators are peptides enhancing the recognition of unwanted neoplasms by immune cells and/or cytotoxic effects of immune cells containing one or more mutations and/or amino acid substitutions of the ras proteins, the p53 protein, the EGF-receptor protein, fusion peptides and/or fusion proteins, the retinoblastoma protein, proteins coded by
10 oncogenes and/or protooncogenes and/or proteins coded by anti-oncogenes and/or tumor suppressor genes.

- In yet another embodiment the immunostimulators are peptides enhancing the recognition of unwanted neoplasms by immune cells and/or cytotoxic effects of immune cells containing one or more mutations and/or amino acid substitu-
15 tions caused by gene rearrangements and/or gene translocations.

In yet another embodiment the immunostimulators are peptides enhancing the recognition of unwanted neoplasm by immune cells and/or cytotoxic effects of immune cells derived from proteins differing in the target cell by one or more amino acids from the proteins expressed by other cells in the same organism.

- 20 In yet another preferred embodiment the immunostimulators are peptides enhancing the recognition of unwanted neoplasm by immune cells and/or cytotoxic effects of immune cells derived from viral antigens and/or coded by viral nucleic acids.

- In yet another embodiment the immunostimulators are peptides derived from
25 proteins expressed in a diseased organ but not in the nervous system, muscle, hematopoietic system or other organs essential for survival. Diseased organs are e.g. prostate, ovary, breast, melanine producing cells and the like.

In yet another embodiment the immunostimulator is a peptide containing one or more amino acids differing between a protein in the target cell from the

other cells within an organism, tumor cell extracts, tumor cell lysates and/or adjuvants.

In yet another embodiment the immunostimulator are fusion cells of dendritic and tumor cells or are dendritic cells. These fusion cells are hybridoma cells derived from a mixture of dendritic cells and tumor cells. Dendritic cells are generated e.g. by treatment of PBMC with GM-CSF and IL-4 or a mixture of GM-CSF, IL-4 and IFN- γ or FLT-3 ligand. Fusion of dendritic cells with tumor cells can be achieved e.g. using PEG (polyethylene glycole) or electrofusion (Hayashi, T., et al. 2002, Parkhust, M.R. 2003, Phan, V., 2003).

- 10 In yet another preferred embodiment the immunostimulator is an antagonist of factors negatively influencing the function of the immune system. These factors are e.g. TGF-beta (tumor growth factor beta), VEGF (vascular endothelial growth factor), PGE₂(prostaglandin E₂), IL 10 (interleukine10).

In yet another embodiment the immunostimulator is a vaccine.

15 Vaccines

Vaccines according to this invention comprise but are not limited to substance in a pharmaceutical acceptable carrier selected from the group of whole (irradiated) tumor cells, ASI (active specific immunization) with e.g. Newcastle Disease Virus (NDV) modified tumor cell vaccine (Schneider, T. et al. 2001), tumor cell lysates.

In one preferred embodiment the vaccines are peptides, peptides combined with cytokines (e.g. IL-2, IL-12, GM-CSF) or peptides combined with adjuvants (e.g. Incomplete Freund's adjuvant, QS21).

- 25 In yet another embodiment of vaccination recombinant virus constructs that encode cancer antigen(s) are part of e.g. adenovirus, vaccinia, fowlpox and/or avipox.

In yet another embodiment the vaccine is naked DNA encoding cancer antigen(s).

In yet another embodiment the vaccines are dendritic cells, dendritic cells loaded with peptides derived from cancer antigens, dendritic cells transfected
5 with recombinant viruses or RNA, DNA and/or cDNA encoding different tumor antigens, dendritic cells pulsed with tumor lysates and/or dendritic cells fused with whole tumor cells.

For further vaccines see also Jäger, E. et al. 2003.

Antagonist

10 In a preferred embodiment of this invention the immunostimulator is an antagonist of factors negatively influencing the function of the immune system.

An "antagonist" as used herein is any substance inhibiting the production of e.g. a cytokine and/or the effect of cytokines. Examples for cytokines negatively influencing the immune systems are e.g. TGF-beta, VEGF, IL-10, PGE-
15 E₂. The inhibition in one embodiment works by binding the cytokine to a binding protein, to a receptor or to a part of this receptor, by binding the cytokine with an antibody, a low molecular substance inhibiting the cytokine or its production, or by inhibiting the signal pathway of said cytokine, e.g. by inhibiting the receptors of these cytokines or any other link downstream in the
20 activation cascade of cytokines.

More details are given for the preferred embodiment of TGF-beta antagonists, which can be transferred to the cytokines described above as well.

Antagonist of the immune system as used herein is any substance or method inhibiting the activity of the immune system.

25 Low molecular substance

"Low molecular substances" or "small molecules" herein comprise substances

with a molecular weight of less than about 10.000 Da and more than about 1 Da of organic or anorganic origin.

TGF-beta Antagonists

In a preferred embodiment the at least one immunostimulator of the pharmaceutical composition of this invention is an TGF-beta antagonist.

TGF-beta (tumor growth factor beta) in the context of this invention comprises all subclasses of TGF-beta, preferred subclasses are TGF-beta 1, TGF-beta 2 and TGF-beta 3.

In the context of this invention a TGF-beta antagonist is any substance inhibiting the function of TGF-beta which means that any effect that is induced by TGF-beta is inhibited.

In preferred embodiments TGF-beta antagonists are substances inhibiting the production of TGF-beta, are substances binding TGF-beta and/or are substances inhibiting the function of TGF-beta downstream its activation cascade. For more details about TGF-beta antagonists see also Wojtowicz-Praga, S. 2003 herein incorporated by reference. Examples for TGF-beta antagonists are given in Example 7.

In one embodiment of TGF-beta antagonists inhibiting the production of TGF-beta are oligonucleotides and/or their active derivatives hybridising with an area of the messenger RNA (mRNA) of TGF-beta and/or the DNA encoding TGF-beta and by this inhibit the production of TGF-beta.

In yet another embodiment the substance inhibiting the production of TGF-beta is a peptide, a protein and/or a small molecule (e.g. tranilast (N-[3,4-dimethoxycinnamoyl]-anthranilic acid) (Wilkenson, K.A. 2000).

In yet another embodiment TGF-beta antagonists are receptors and/or parts of it binding TGF-beta and in that way inhibit the function of TGF-beta.

In yet another embodiment the TGF antagonist is an antibody and/or parts of it binding TGF-beta and by this inhibit the function of TGF-beta. Those antibodies are commercially available, see e.g. R & D Systems, Inc. The production of those antibodies is well-known in the art. Animals such as e.g. chicken,
5 mouse, rabbits, goat, are immunized with purified human TGF-beta. IgY then is purified with e.g. affinity chromatography as described for example by Cooper, H.M. 1995. In yet other embodiments the TGF-beta antibodies are further modified e.g. bitionylated.

In a more preferred embodiment the TGF-beta antibodies are humanized anti-
10 bodies. For more details about humanized antibodies see also Carrington 1998.

In yet another embodiment the TGF antagonist is a protein and/or peptide binding to TGF-beta and by this inhibiting the function of TGF-beta. Preferred
15 embodiments of these peptides are e.g. Latency-associated peptides and can inhibit all three isoforms of TGF-beta (TGF-beta 1, TGF-beta 2 and TGF-beta 3).

In another embodiment the TGF-beta inhibitor is a protein, peptide or a small molecule inhibiting the function of the TGF-beta receptor, acting extracellular or intracellular.

In yet other embodiment the TGF-beta antagonists comprise, proteins, peptides, antibodies and/or small molecules which inhibit the TGF-beta activity by
20 inhibiting any link downstream the TGF-beta cascade of activation.

Nucleic Acid/Oligonucleotides

In a preferred embodiment of this invention the antagonists of a peptide, cytokine and/or receptor are nucleic acids.

25 The terms "nucleic acid" and "oligonucleotide" refer to multiple nucleotides (i.e. molecules comprising a sugar (e.g. ribose or deoxyribose) linked to a phosphate group and to an variable organic base, which is either a substituted

pyrimidine (e.g. cytosine (C), thymine (T) or uracil (U)) or a substituted purine (e.g. adenine (A) or guanine (G)) or a modification thereof. As used herein, the terms refer to oligoribonucleotides as well as oligodeoxyribonucleotides.

The terms shall also include oligonucleosides (i.e. a oligonucleotide without the

5 phosphate) and any other organic base containing polymer. The nucleic acids may be double-stranded or single-stranded. Double-stranded molecules may be more stable in vivo, while single-stranded molecules may have increased activity. In one embodiment the nucleotides have length between about 6 and about 100 nucleotides in yet another embodiment the nucleotides have length
10 of about 8 to about 40 nucleotides respectively from about 12 to about 32 nucleotides.

As used herein with respect to linked units of a nucleic acid, "linked" or "linkage" means two entities are bound to one another by any physicochemical means. Any linkage known to those of ordinary skill in the art, covalent or
15 noncovalent, is embraced. Natural linkages, which are those ordinarily found in nature connecting the individual units of a nucleic acid, are most common. The individual units of a nucleic acid may be linked, however, by synthetic or modified linkages.

In one embodiment the respective ends of this linear polymeric structure can
20 be further joined to form a circular structure. However, open linear structures are generally preferred. Within the oligonucleotides structure, the phosphate groups are commonly referred to as forming the internucleoside backbone of the oligonucleotide. The normal linkage or backbone of RNA and DNA is a 3' to 5' phosphodiester linkage.

25 In one embodiment the terms "nucleic acids", "nucleotides", "oligonucleotides" respectively "antisense oligonucleotides" are substances stimulating the function of the immune system and/or the immune cells and/or are antagonists of TGF-beta as described herein. In preferred embodiments they comprise DNA- or RNA-fragments coding for TGF-beta and/or its receptors, VEGF and/or its
30 receptors, interleukin 10 (IL-10) and/or its receptors, PGE₂ and/or its recep-

tors or are the respective antisense nucleotides and/or are ribozymes.

In still other embodiments, the nucleic acids are not antisense nucleic acids, meaning that they do not function by binding to complementary genomic DNA or RNA species within a cell and thereby inhibiting the function of said genomic

5 DNA or RNA species.

In one embodiment the sequences comprises the sequences as described in the Patents EP 069 53 54 and EP 1008649 as well as those of the international patent applications published under No. WO 01/68 146, WO98/33904 and WO 99/63975 herein incorporated by reference. TGF-beta antisense oligonucleo-
10 tides in one preferred embodiment include at least one sequence set forth as SEQ ID NOs: 1-127.

Oligonucleotide-Modifications:

Oligonucleotides or nucleic acids includes oligonucleotides having non-naturally-occurring portions with similar function. Such modified or substituted
15 oligonucleotides are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target (e.g. protein), altered intracellular localization and increased stability in the presence of nucleases. Modifications of the oligonucleotides as used herein comprises any chemical modifications of the sugar, the
20 base moiety and/or the internucleoside linkage.

In one embodiment nucleic acids or oligonucleotides with a covalently modified base and/or sugar include for example nucleic acids having backbone sugars which are covalently attached to low molecular weight organic groups other than a hydroxyl group at the 3' and/or 2' position and other than a phosphate
25 group at the 5' position. Thus modified nucleic acids may include a 2'-O-alkylated ribose group. In yet another embodiment modified nucleic acids include sugars such as arabinose instead of ribose. Thus the nucleic acids may be heterogeneous in backbone composition thereby containing any possible combination of polymer units linked together such as peptide-nucleic acids

(which have amino acid backbone with nucleic acid bases). In some embodiments the nucleic acids are homogeneous in backbone composition.

The substituted purines and pyrimidines of the nucleic acids include standard purines and pyrimidines such as cytosine as well as base analogs such as C-S

5 propyne substituted bases (Wagner et al., Nature Biotechnology 14:840-844, 1996). Purines and pyrimidines include but are not limited to adenine, cytosine, guanine, thymine, 5-methylcytosine, 2-aminopurine, 2-amino-6-chloropurine, 2,6-diaminopurine, hypoxanthine, and other naturally and non-naturally occurring nucleobases, substituted and unsubstituted aromatic moie-

10 ties.

The single nucleotides in each oligonucleotide or polynucleotide polymer may contain the same modifications, may contain combinations of these modifications, or may combine these modifications with phosphodiester linkages. Methods of rendering oligonucleotide or polynucleotide polymers nuclease

15 resistant include, but are not limited to, covalently modifying the purine or pyrimidine bases. For example, bases may be methylated, hydroxymethylated, or otherwise substituted (e.g., glycosylated) such that the oligonucleotides or polynucleotides are rendered substantially acid and nuclease resistant.

In a preferred embodiment, at least one end-block on the oligonucleotide is a

20 biotin, biotin analog, avidin, or avidin analog. These molecules have the ability to both block the degradation of the protected oligonucleotide or polynucleotide and provide means for high affinity attachment of the modified nucleic acids to the solid support. Avidin and biotin derivatives which can be used to prepare the reagents of this invention include streptavidin, succinylated avidin,

25 monomeric avidin, biocytin (biotin-epsilon-N-lysine), biocytin hydrazide, amine or sulfhydryl derivatives of 2-iminobiotin and biotinyl-epsilon-aminocaproic acid hydrazide. Additional biotin derivatives, such as biotin-N-hydroxysuccinimide ester, biotinyl-epsilon-aminocaproic acid-N-hydroxysuccinimide ester, sulfosuccinimidyl 6-(biotin amido)hexanoate, N-

30 hydroxysuccinimideiminobiotin, biotinbromoacetylhydrazide, p-diazobenzoyl

biocytin and 3-(N-maleimidopropionyl)biocytin, can also be used as end-blocking groups on the polynucleotides of the present invention.

In another embodiment the ring structure of the ribose group of the nucleotides in the modified oligonucleotide or polynucleotide has an oxygen in the ring structure substituted with N-H, N-R (with R being an alkyl or aryl substituent), S and/or methylene.

In yet another embodiment the base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an oligonucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative United States patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos.: 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found in Nielsen et al., Science, 1991, 254, 1497-1500.

Further modified oligonucleotide backbones include, for example, phosphorothioates; chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-aminophosphoamidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having norm 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts, and free acid forms are also included.

Modification of the ends/ irregular modifications

In embodiments at least one nucleotide of an oligonucleotide is modified as described in one of the modifications above., in yet another embodiment the modification is only can

- 5 In yet another embodiment both of these modifications of the oligonucleotide are combined.

In preferred embodiments the 1 to about 12 or 1 to about 8 or 1 to about 4 or 1 to about 2 oligonucleotides and/or nucleotide linkages at the 3' and/or 5' end of the oligonucleotide are modified as described above.

- 10 In yet another embodiment the oligonucleotides of this invention hybridizing with a target (e.g. TGF-beta or its subtypes, VEGF, IL-10, PGE₂). Comprising in the context of this invention means that one of the oligonucleotides of the sequence listing is part of the antisense oligonucleotide of the respective m-RNA. In one embodiment even the complete antisense oligonucleotide of the
- 15 m-RNA of the target is an immunostimulator in the meaning of this invention. IN yet another embodiment any part of the antisense m-RNA of a target negatively influencing the function of the immune system is within the scope of this invention. This means that oligonucleotides of the sequence listing that have additionally oligonucleotides of the sequence of the respective antisense m-
- 20 RNA with about 1 to about 1000 nucleotides, from about 1 to about 500, from about 1 to about 100, from about 1 to about 50 from about 1 to about 20, from about 1 to about 10, from about 1 to about 5 or from about 1 to about 2 nucleotides bound to at least one of the, 3' and/or 5' end; I a preferred embodiment on at least one of the 2' and/or 5' end, are still within the scope of
- 25 this invention..

The nucleotide sequence of targets of factors negatively influencing the function of immune cells and/or the immune system, as well as the respective antisense sequences, are known to persons skilled in the art. In a preferred embodiment the targets are selected from the group of m-RNA of TGF-beta 1,

TGF-beta 2 and/or TGF-beta 3. The sequence of the antisense m-RNA of TGF-beta-1, TGF beta-2, TGF-beta-3, interleukine 10, VEGF and PGE₂ synthase is given in example 6.

Synthesis

5 For use in the instant invention, the nucleic acids can be synthesized de novo using any of a number of procedures well known in the art. Such compounds are referred to as 'synthetic nucleic acids.' For example, the b-cyanoethyl phosphoramidite method (Beaucage et al. 1981); nucleoside H-phosphonate method (Garegg et al. 1986, Froehler et al. 1986, Garegg et al. 1986, Gaffney
10 et al., 1988). These chemistries can be performed by a variety of automated oligonucleotide synthesizers available in the market.

Alternatively, nucleic acids can be produced on a large scale in plasmids, (see, e.g., Sambrook, et al. 1989) and separated into smaller pieces or administered whole. Nucleic acids can be prepared from existing nucleic acid sequences
15 (e.g., genomic or cDNA) using known techniques, such as those employing restriction enzymes, exonucleases or endonucleases. Nucleic acids prepared in this manner are referred to as isolated nucleic acids. The term "antineoplastic nucleic acid" encompasses both synthetic and isolated antineoplastic nucleic acids.

20 Modified backbone nucleic acids, such as those having phosphorothioates bonds may be synthesized using automated techniques employing, for example, phosphoramidate or H-phosphonate chemistries. Aryl- and alkyl-phosphonates can be made, e.g., as described in U.S. Pat. No. 4,469,863. Alkylphosphotriesters, in which the charged oxygen moiety is alkylated as
25 described in U.S. Pat. No. 5,023,243 and European Patent No. 092,574, can be prepared by automated solid phase synthesis using commercially available reagents. Methods for making other nucleic acid backbone modifications and substitutions have been described (Uhlmann et al. 1990, Goodchild, 1990).

Another type of modified backbone, useful according to the invention, is a

peptide nucleic acid. The backbone is composed of aminoethylglycine and supports bases which provide the nucleic acid character. The backbone does not include any phosphate and thus may optionally have no net charge. The lack of charge allows for stronger DNA-DNA binding because the charge repul-

5 sion between the two strands does not exist. Additionally, because the backbone has an extra methylene group, the oligonucleotides are enzyme/protease resistant. Peptide nucleic acids can be purchased from various commercial sources, e.g., Perkin Elmer, or synthesized de novo.

The nucleic acids having backbone modifications useful according to the inven-
10 tion in some embodiments are S- or R-chiral antineoplastic nucleic acids. An "S chiral antineoplastic nucleic acid" as used herein is an antineoplastic nucleic acid wherein at least two nucleotides have a backbone modification forming a chiral center and wherein a plurality of the chiral centers have S chirality. An "R chiral antineoplastic nucleic acid" as used herein is an antineoplastic nucleic
15 acid wherein at least two nucleotides have a backbone modification forming a chiral center and wherein a plurality of the chiral centers have R chirality. The backbone modification may be any type of modification that forms a chiral center. The modifications include but are not limited to phosphorothioate, methylphosphonate, methylphosphorothioate, phosphorodithioate, p-ethoxy,
20 2'-O-Me and combinations thereof.

Phosphorothioates may be synthesized using automated techniques employing either phosphoramidate or H-phosphonate chemistries. Aryl- and alkylphosphonates can be made, e.g., as described in U.S. Pat. No. 4,469,863; and alkylphosphotriesters (in which the charged oxygen moiety is alkylated as
25 described in U.S. Pat. No. 5,023,243 and European Patent No. 092,574) can be prepared by automated solid phase synthesis using commercially available reagents. Methods for making other DNA backbone modifications and substitutions have been described (Uhlmann, E. et al. 1990, Goodchild, J. 1990).

Other sources of nucleic acids useful according to the invention include stan-
30 dard viral and bacterial vectors, many of which are commercially available. In

its broadest sense, a "vector" is any nucleic acid material which is ordinarily used to deliver and facilitate the transfer of nucleic acids to cells. The vector as used herein may be an empty vector or a vector carrying a gene which can be expressed. In the case when the vector is carrying a gene the vector generally

5 transports the gene to the target cells with reduced degradation relative to the extent of degradation that would result in the absence of the vector. In this case the vector optionally includes gene expression sequences to enhance expression of the gene in target cells such as immune cells, but it is not required that the gene be expressed in the cell.

10 As used herein, the term "neoplasm" means new and abnormal growth or formation of tissue and/or blood cells in the body of a organism. The unwanted neoplasms include, but are not limited to, solid tumors; blood born tumors such as leukemias such as acute or chronic myelotic or lymphoblastic leukemia; tumor metastasis; benign tumors, for example hemangiomas, acoustic
15 neuromas, neurofibromas, trachomas, and pyogenic granulomas; pre-malignant tumors; rheumatoid arthritis; psoriasis;

Astracytoma, Acoustic neuroma, blastoma, Ewing's tumor, astracytoma, craniopharyngloma, ependymoma, medulloblastoma, glioma, hemangloblastoma, Hodgkins-lymphoma, medullablastoma, leukaemia, mesothelioma, neuroblas-
20 toma; neurofibroma, non-Hodgkins lymphoma, pinealoma, retinoblastoma, retinoblastoma, sarcoma (including angiosarcoma, chondrosarcoma, endothelial sarcoma, fibrosarcoma, leiomyosarcoma, liposarcoma, lymphangioendothelial sarcoma, lymphangiosarcoma, melanoma, meningioma, myosarcoma, oligodendroglioma, osteogenic sarcoma, osteosarcoma), seminoma, trachomas,
25 Wilm's tumor,

or is selected from the group of bile duct carcinoma, bladder carcinoma, brain tumor, breast cancer, bronchogenic carcinoma, carcinoma of the kidney, cervical cancer, choriocarcinoma, cystadenocarcinome, embrional carcinoma, epithelial carcinoma, esophageal cancer, cervical carcinoma, colon carcinoma,
30 colorectal carcinoma, endometrial cancer, gallbladder cancer, gastric cancer,

head cancer, liver carcinoma, lung carcinoma, medullary carcinoma, neck cancer, non-small-cell bronchogenic/lung carcinoma, ovarian cancer, pancreas carcinoma, papillary carcinoma, papillary adenocarcinoma, prostata cancer, small intestine carcinoma, prostate carcinoma, rectal cancer, renal cell carcinoma, skin cancer, small-cell bronchogenic/lung carcinoma, squamous cell carcinoma, sebaceous gland carcinoma, testicular carcinoma, uterine cancer,

Antineoplastic agents

Pharmaceutical compositions of this invention beside the immunostimulator comprise at least one substance inhibiting cell proliferation and/or inducing cell.

An "antineoplastic chemotherapeutic agent" as used herein is a substance inhibiting cell proliferation and/or inducing cell death and in a preferred embodiment further inhibits the formation of metastases not by stimulating the immune cells and/or the function of the immune system as described herein.

The term antineoplastic chemotherapeutic agent comprises, but is not limited to antineoplastic agents, antineoplastic supplementary potentiating agents and radioactive agents. Examples for this group are given herein.

In a preferred embodiment the antineoplastic chemotherapeutic agent is selected from the group of ACNU, BCNU, CCNU, cisplatin, cyclophosphamide, pegylated liposomal doxorubicin (cealyx®), 5-fluorodeoxyuridine, 5-fluorouracil, 5-fluorouridine, gemcitabine, procarbazine, taxol, taxotere, temozolomid, vinblastine, vincristine.

Synonyms for ACNU are 3-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea hydrochloride, CS-439 HCl, Nidran hydrochloride, Nimustine Hydrochloride, NSC-245382,

BCNU is Bischloroethylnitrosourea, the chemical name is N,N'-bis(2-chloroethyl)-N-nitroso-urea, other names are BiCNU, carmustine.

CCNU is 1-(2-Chloroethyl)-3-cyclohexyl-1-nitroso-urea. Synonyms are N-(2-chloroethyl)-N'-cyclohexyl-N-nitroso-urea, Belustine, Cee NU, Chloroethylcyclohexylnitroso-urea, ICIG 1109, Lomustine, NSC.79037.

One chemical name for temozolomide is 3,4-dihydro-3-methyl-4-oxoimidazo-
5 >5,1d'1,2,3,4-tetrazin-8-carboximide. Other names for temozolomide are temodal, methazolastone, CCRG81045, SCH52365, NSC362856, M&B39836.

Synonyms for teniposide are: 4'-Demethylepipodophyllotoxin, 9-(4,6-O-2-thienylidene-b-D-glucopyranoside), Epipodophyllotoxin, EPT, Teniposide VM-26, VM 26, 5,8,8a,9-Tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl)-9-{[4,6-O-
10 (2-thienylmethylene)-b-D-glucopyranosyl]oxy}furo[3',4':6,7]naphtho[2,3-d]-1,3-dioxol-6(5aH)-one.

In other embodiments the at least one immunostimulator of this invention is combined with at least one antineoplastic agent selected from the following group:

15 Acivicin; Aclarubicin; Acodazole Hydrochloride; Acronine; Adozelesin; Adriamycin; Aldesleukin; Altretamine; Ambomycin; Ametantrone Acetate; Aminoglutethimide; Amsacrine; Anastrozole; Anthramycin; Asparaginase; Asperlin; Azacitidine; Azetepa; Azotomycin; Batimastat; Benzodepa; Bicalutamide; Bisantrone Hydrochloride; Bisnafide Dimesylate; Bizelesin; Bleomycin Sulfate;
20 Brequinar Sodium; Bropiramine; Busulfan; Cactinomycin; Calusterone; Caracemide; Carbetimer; Carboplatin; Carmustine; Carubicin Hydrochloride; Carzelesin; Cedefingol; Cetuximab; Chlorambucil; Cirolemycin; Cisplatin; Cladribine; Crisnatol Mesylate; Cyclophosphamide; Cytarabine; Dacarbazine; DACA (N-[2-(Dimethyl-amino)ethyl]acridine-4-carboxamide); Dactinomycin;
25 Daunorubicin Hydrochloride; Daunomycin; Decitabine; Dexormaplatin; Dezaguanine; Dezaguanine Mesylate; Diaziquone; Docetaxel; Doxorubicin; Doxorubicin Hydrochloride; Droloxifene; Droloxifene Citrate; Dromostanolone Propionate; Duazomycin; Edatrexate; Eflornithine Hydrochloride; Elsamitrucin;

Enloplatin; Enpromate; Epiropidine; Epirubicin Hydrochloride; Erbulozole; Erlotinib; Erorubicin Hydrochloride; Estramustine; Estramustine Phosphate Sodium; Etanidazole; Ethiodized Oil I 131; Etoposide; Etoposide Phosphate; Etoprine; Fadrozole Hydrochloride; Fazarabine; Fenretinide; Floxuridine;

5 Fludarabine Phosphate; Fluorouracil; 5-FdUMP; Flurocitabine; Fosquidone; Fostriecin Sodium; Gefitinib; Gemcitabine; Gemcitabine Hydrochloride; Gold Au 198 ; Hydroxyurea; Idarubicin Hydrochloride; Ifosfamide; Ilmofofine; Imatinib mesylate; Interferon Alfa-2a; Interferon Alfa-2b ; Interferon Alfa-n1; Interferon Alfa-n3; Interferon Beta-I a; Interferon Gamma-I b; Iproplatin;

10 Iressa; Irinotecan Hydrochloride; Lanreotide Acetate; Letrozole; Leuprolide Acetate; Liarozole Hydrochloride; Lometrexol Sodium; Lomustine; Losoxantrone Hydrochloride; Masoprocol; Maytansine; Mechlorethamine Hydrochloride; Megestrol Acetate; Melengestrol Acetate; Melphalan; Menogaril; Mercaptopurine; Methotrexate; Methotrexate Sodium; Metoprine; Meturedopa;

15 Mitindomide; Mitocarcin; Mitocromin; Mitogillin; Mitomalcin; Mitomycin; Mitosper; Mitotane; Mitoxantrone Hydrochloride; Mycophenolic Acid; Nocodazole; Nogalamycin; Ormaplatin; Oxisuran; Paclitaxel; Pegaspargase; Peliomycin; Pentamustine; Peplomycin Sulfate; Perfosfamide; Pipobroman; Puposulfan; Piroxantrone Hydrochloride; Plicamycin; Plomestane; Porfimer Sodium; Porfiro-

20 romycin; Prednimustine; Procarbazine Hydrochloride; Puromycin; Puromycin Hydrochloride; Pyrazofurin; Riboprine; Rituximab; Rogletimide; Safinol; Saffin-gol Hydrochloride; Semustine; Simtrazene; Sparfosate Sodium; Sparsomycin; Spirogermanium Hydrochloride; Spiromustine; Spiroplatin; Streptonigrin; Streptozocin; Strontium Chloride Sr 89; Sulofenur; Talisomycin; Taxane;

25 Taxoid; Tecogalan Sodium; Tegafur; Teloxantrone Hydrochloride; Temoporfin; Teniposide; Teroxirone; Testolactone; Thiamiprine; Thioguanine; Thiotepa; Thymitaq; Tiazofurin; Tirapazamine; Tomudex; TOP-53; Topotecan Hydrochloride; Toremifene Citrate; Trastuzumab; Trestolone Acetate; Triciribine Phosphate; Trimetrexate; Trimetrexate Glucuronate; Triptorelin; Tubulozole

30 Hydrochloride; Uracil Mustard; Uredopa; Vapreotide; Verteporfin; Vinblastine; Vinblastine Sulfate; Vincristine; Vincristine Sulfate; Vindesine; Vindesine Sulfate; Vinepidine Sulfate; Vinglycinat. Sulfate; Vinleurosine Sulfate; Vinorelbine

Tartrate; Vinrosidine Sulfate; Vinzolidine Sulfate; Vorozole; Zeniplatin; Zinostatin; Zorubicin Hydrochloride; 2-Chlorodeoxyadenosine; 2'-Deoxformycin; 9-aminocamptothecin; raltitrexed; N-propargyl-5,8-dideazafolic acid; 2-chloro-2'-arabino-fluoro-2'-deoxyadenosine; 2-chloro-2'-deoxyadenosine; anisomycin; trichostatin A; hPRL-G129R; CEP-751; linomide.

Other anti-neoplastic agents include:

20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplastic; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurin; beta lactam derivatives; beta-alethine; betacarmycin B; betulonic acid; bFGF inhibitor; biclutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; broprimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives (e.g., 10-hydroxy-camptothecin); canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentantraquinones; cycloplatin; cypemycin; cytarabine ocfosphate; cytolytic factor; cytostatin; dactinomycin; decitabine; dehydrodidemnin B; deslorelin; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorsper-

mine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl spiro-
mustine; discodermolide; docosanol; dolasetron; doxifluridine; droloxifene;
dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab;
eflornithine; elemene; emitefur; epirubicin; epothilones including desoxye-
5 pothilones (A, R.dbd.H; B, R.dbd.Me); epithilones; epristeride; estramustine
analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide;
etoposide 4'-phosphate (etopofos); exemestane; fadrozole; fazarabine; fen-
retinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fluda-
rabin; fluorodaunorubicin hydrochloride; forfenimex; formestane; fostriecin;
10 fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix;
gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam;
heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin;
idoxifene; idramantone; ilmofofosine; ilomastat; imidazoacridones; imiquimod;
immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor;
15 interferon agonists; interferons; interleukins; lobenguane; iododoxorubicin;
ipomeanol, 4-; irinotecan; iroplact; irsogladine; isobengazole; isohomohalicon-
drin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreo-
tide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leuke-
mia inhibiting factor; leukocyte alpha interferon;
20 leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear
polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum com-
pounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine;
losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofyl-
line; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol;
25 maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril;
merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor;
mifepristone; miltefosine; mirimostim; mismatched double stranded RNA;
mithracin; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mito-
toxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramo-
30 stim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl
lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene
inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer

agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; 5 nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; ox-aunomycin; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; 10 pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pento-statin; pentozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; podo- 15 phyllotoxin; porfimer sodium; porfiromycin; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf 20 antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safinol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived 25 inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division 30 inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; taz-

arotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thalidomide; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene dichloride; topotecan; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; tricyriline; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; zinostatin stimalamer.

Antineoplastic Supplementary Potentiating Agents:

Tricyclic anti-depressant drugs (e.g., imipramine, desipramine, amitriptyline, clomipramine, trimipramine, doxepin, nortriptyline, protriptyline, amoxapine and maprotiline); non-tricyclic anti-depressant drugs (e.g., sertraline, trazodone and citalopram); Ca.sup.++ antagonists (e.g., verapamil, nifedipine, nitrendipine and caroverine); Calmodulin inhibitors (e.g., prenylamine, trifluoroperazine and clomipramine); Amphotericin B; Triparanol analogues (e.g., tamoxifen); antiarrhythmic drugs (e.g., quinidine); antihypertensive drugs (e.g., reserpine); Thiol depleters (e.g., buthionine and sulfoximine) and Multiple Drug Resistance reducing agents such as Cremaphor EL. The compounds of the invention also can be administered with cytokines such as granulocyte colony stimulating factor.

Antiproliferative agent: Piritrexim Isethionate.

Radioactive agents:

Fibrinogen I 125; Fludeoxyglucose F 18 ; Fluorodopa F 18; Insulin I 125; Insulin I 131; Iobenguane I 123; Iodipamide Sodium I 131; Iodoantipyrine I 131; Iodocholesterol I 131; Iodohippurate Sodium I 123; Iodohippurate Sodium I

125; Iodohippurate Sodium I 131; Iodopyracet I 125; Iodopyracet I 131; Iofetamine Hydrochloride I 123; Iomethin I 125; Iomethin I 131; Iothalamate Sodium I 125 Iothalamate Sodium I 131; Iotyrosine I 131; Liothyronine I 125; Liothyronine I 131; Merisoprol Acetate Hg 197; Merisoprol Acetate Hg 203; 5 Merisoprol Hg 197; Selenomethionine Se 75; Technetium Tc 99m Antimony Trisulfide Colloid; Technetium Tc 99m Bicisate; Technetium Tc 99m Disofenin; Technetium Tc 99m Etidronate; Technetium Tc 99m Exametazime; Technetium Tc 99m Furifosmin; Technetium Tc 99m Gluceptate; Technetium Tc 99m Lidofenin; Technetium Tc 99m Mebrofenin; Technetium Tc 99m Medronate; 10 Technetium Tc 99m Medronate Disodium; Technetium Tc 99m Mertiatide; Technetium Tc 99m Oxidronate; Technetium Tc 99m Pentetate; Technetium Tc 99m Pentetate Calcium Trisodium; Technetium Tc 99m Sestamibi; Technetium Tc 99m Siboroxime; Technetium Tc 99m Succimer; Technetium Tc 99m Sulfur Colloid; Technetium Tc 99m Teboroxime; Technetium Tc 99m Tetrofosmin; 15 Technetium Tc 99m Tiatide; Thyroxine I 125; Thyroxine I 131; Tolpovidone I 131; Triolein I 125; Triolein I 131.

The term antineoplastic chemotherapeutic agents also includes nucleic acid molecules for the inhibition of angiogenesis and inductors of the aggregation of tubulin.

20 Active derivatives of the antineoplastic chemoptherapeutic agents as well as prodrugs are also part of this invention.

Since a common but tolerable side effect of antineoplastic agents is nausea and vomiting it is obvious to someone skilled in the art, that these effects can be avelliated by administering an anti-emetic in conjunction with the antineo- 25 lastic agent inducing nausea and/or vomiting. E.g. Ondansetron may be given p.o. in a dose of about 8 mg about 30 minutes before the nausea/vomiting inducing antineoplastic agent is administerd. Of ourse other anti emtics such as Hasaldol, Benadryl, and Ativan may also be used as needed.

Synthesis of antineoplastic agents

The antineoplastic chemotherapeutic agent of this invention are commercial available. For the synthesis of e.g. temozolomid see for example Stevens et al. 1984 or Wang et. al 1994.

5 Radiation

Radiation is applied in dosages of about 1 Gy to about 100 Gy, more preferred from about 20 to about 80 Gy and most preferred, e.g. for the treatment of astrocytoms and glioms from about 40 to about 60 Gy.

10 The dosage in preferred embodiments is fractionated which means that, from about 0.1 to about 10 Gy or from about 1 Gy to about 5 Gy or from about 1 Gy tot about 2 Gy are applied in one session which is repeated several times during about 1 to about 20 weeks, about 2 to about 1 weeks or 4 to about 8 weeks. The antagonist and or the substance inhibiting cell proliferation and/or inducing cell death of this invention can be administered before, after or to-
15 gether with the radiation. One cycle of radiation therapy as well as several cacle of radiation are possible, dependent of the reduction of tumor size.

The radiation usually is performed with ^{60}Co . Radiation with neutrons, protons, negative pi-mesones or neutrone capture are applicable as well.

20 It is clear to someone skilled in the art, that the dosage further is dependant from the size of the tumor, the figure of the patient and the kind of radiation applied. In special embodiments the dosage is about 2 to about 100 fold higher or lower as described above also dependant from the number of frac-
tions the dosage is applied with.

Application

25 In one embodiment the combination of at least one immunostimulator and at least one antineoplastic agent is useful in the treatment of unwanted neoplasms such as but not limited to bile duct carcinoma, bladder carcinoma,

brain tumor, breast cancer, bronchogenic carcinoma, carcinoma of the kidney, cervical cancer, choriocarcinoma, cystadenocarcinoma, embryonal carcinoma, epithelial carcinoma, esophageal cancer, cervical carcinoma, colon carcinoma, colorectal carcinoma, endometrial cancer, gallbladder cancer, gastric cancer, head cancer, liver carcinoma, lung carcinoma, medullary carcinoma, neck cancer, non-small-cell bronchogenic/lung carcinoma, ovarian cancer, pancreas carcinoma, papillary carcinoma, papillary adenocarcinoma, prostata cancer, small intestine carcinoma, prostate carcinoma, rectal cancer, renal cell carcinoma, skin cancer, small-cell bronchogenic/lung carcinoma, squamous cell carcinoma, sebaceous gland carcinoma, testicular carcinoma, uterine cancer.

acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; pre-malignant tumors, astracytoma, blastoma, Ewing's tumor, craniopharyngloma, ependymoma, medulloblastoma, glioma, hemangioblastoma, Hodgkins-lymphoma, medullablastoma, leukaemia, mesothelioma, neuroblastoma, neurofibroma, non-Hodgkins lymphoma, pinealoma, retinoblastoma, retinoblastoma, sarcoma (including angiosarcoma, chondrosarcoma, endothelial sarcoma, fibrosarcoma, leiomyosarcoma, liposarcoma, lymphangioendothelial sarcoma, lymphangiosarcoma, melanoma, meningioma, myosarcoma, oligodendroglioma, osteogenic sarcoma, osteosarcoma), seminoma, trachomas, Wilm's tumor.

In another embodiment the composition of at least one agonist and at least one antineoplastic agent may be used in combination with other procedures for the treatment of diseases. For example, a tumor may be treated conventionally with surgery and/or radiation and then the composition of an immunostimulator and antineoplastic chemotherapeutic agent according to this invention may be subsequently administered to the patient to extend the dormancy of micrometastases and to stabilize respectively reduce any residual unwanted neoplasm.

In a preferred embodiment a combination of at least one antineoplastic agent and at least one antagonist is administered to a site likely to harbor a metas-

tatic lesion (that may or may not be clinically discernible at the time). A sustained release formulation implanted specifically at the site (or the tissue) where the metastatic lesion is likely to be would be suitable in these latter instances.

5 Dosage

The embodiments of the combination of at least one stimulator of the immune cells and/or the immune system and at least one substance inhibiting cell proliferation, and/or inducing cell death is delivered in effective amounts. In general, the term "effective amount" of an antagonist and/or antineoplastic agent
10 refers to the amount necessary or sufficient to realize a desired biologic effect. Specifically, the effective amount is that amount that reduces the rate or inhibits altogether formation of neoplasms. For instance, when the subject bears a tumor, an effective amount is that amount which decreases or eliminates the unwanted neoplasm. Additionally, an effective amount may be that amount
15 which prevents an increase or causes a decrease in new unwanted neoplasms.

The effective amount varies depending upon whether the combination is used in single or multiple dosages. Dosages given in this writing are for adults. It is quite clear to someone skilled in the art, that these dosages have to be adapted if the human being is a child, a person stressed by a further illness or
20 other circumstances.

The effective dosage is dependent also on the method and means of delivery, which can be localized or systemic. For example, in some applications, as in the treatment of skin cancer or ophthalmic cancer the combination preferably is delivered in a topical or ophthalmic carrier.

25 In one embodiment subject doses of the compounds described herein typically range from about 0.1 μ g to about 10 mg per administration, which depending on the application could be given hourly, daily, weekly, or monthly and any other amount of time therebetween. In yet another embodiment the doses range from about 10 μ g to about 5 mg per administration or from about

100 μg to about 1 mg, with 1-10 administrations being spaced hours, days or weeks apart. In some embodiments, however, doses may be used in a range even 2 to 100 fold higher or lower than the typical doses described above.

- ~~In one embodiment of this invention the at least one immunostimulator of a~~
- 5 pharmaceutical composition according to this invention is an antagonist, more preferred an antagonist of TGF-beta and most preferred an antisense oligonucleotide of TGF-beta which is administered in a dose range from about 1 $\mu\text{g/kg/day}$ to about 100 mg/kg/day or from about 10 $\mu\text{g/kg/day}$ to about 10 mg/kg/day or from about 100 $\mu\text{g/kg/day}$ to about 1 mg/kg/day.
10. In a further preferred embodiment of the pharmaceutical composition described herein the at least one immunostimulator, more preferred the TGF-beta antagonist, most preferred the TGF-beta antisense oligonucleotide is administered with a catheter directly into the unwanted neoplasm. The concentrations of these antisense oligonucleotides are from about 0.1 $\mu\text{M/L}$ to
- 15 about 1 M/L, more preferred from about 1 $\mu\text{M/L}$ to about 500 $\mu\text{M/L}$ and even more preferred from about 10 to about 200 $\mu\text{M/L}$ or from about 50 $\mu\text{M/L}$ to about 150 $\mu\text{M/L}$ in a sterile aqueous solution. In yet another preferred embodiment this solution is administered with a flow of about 0.1 $\mu\text{L/min}$ to about 50 $\mu\text{L/min}$ or about 2 $\mu\text{L/min}$ to about 12 $\mu\text{L/min}$ or about 3 $\mu\text{L/min}$ to about
- 20 10 $\mu\text{L/min}$ into the neoplasm.

In yet another embodiment the at least one antineoplastic chemotherapeutic agent is BCNU in combination with at least one immunostimulator and/or radiation is administered in dose range from about 1 mg/m^2 to about 1000 mg/m^2 , more preferred in a dose of about 50 mg/m^2 to about

25 500 mg/m^2 and most preferred in a single dose of about 150 mg/m^2 to 200 mg/m^2 intravenously every 6 weeks. It may be given as a single dose or divided into daily injections such as about 75 mg/m^2 to about 100 mg/m^2 on two successive days.

In yet another embodiment in the treatment of neoplasms the antineoplastic

30 chemotherapeutic agent gemcitabine is administered with at least one im-

munostimulator and/or radiation at a dosage of about 10 mg/m² to about 10 g/m², more preferred from about 100 mg to about 5g/m² and most preferred from about 500 mg/m² to about 2000 mg/m².

~~In another embodiment the dosage of gemcitabine is administered within~~
5 about 10 min to about 120 min, more preferred from about 15 min to about 60 min and most preferred from about 20 min to about 40 min. In yet another embodiment this single dose is administered repeatedly within about 4 to about 10 days, respectively about 5 to about 8 days and most preferred within about 7 days. About 1 to about 8, more preferred about 2 to about 6 most
10 preferred about 3 to about 4 single doses are administered. After this a therapy free interval of about 2 to about 60 days, more preferred about 5 to about 30 days and most preferred from about 10 to about 20 days is applied. Several repetitions of these cycles are possible.

In yet another embodiment at least one antineoplastic chemotherapeutic agent
15 is temozolomide and is administered with a total dose of about 500 to about 1200 mg/m², over a period from about 2 to about 28 consecutive days, more preferable over a period of from about 4 to about 7 consecutive days, and most preferably over a period of about 5 consecutive days. Thus if the total dose is to be about 1000 mg/m² administered over a period of about 5 days,
20 the daily dose for this period is about 200 mg/m²/day. Temozolomide must be administered more than once per day. Preferably dosing regimes would be twice per day, three times per day or four times per day. After a period of about 28 to about 42 days, or about about 28 to about 35 days, or more preferably 28 days, from the first day of temozolomide administration, another
25 administration cycle may be performed.

In yet another embodiment the temozolomide may be administered for a much longer period at reduced dosage. For example, the temozolomide could be administered more than once daily for up to six weeks at a daily dosage of about 50 mg/m²/day to about 150 mg/m² preferably at about 75 mg/m²/day.
30 More preferred these daily doses are split about evenly into two or more doses

to be administered two or more times per day.

In yet another embodiment vinblastin is administered at a dosage of about 0.1 mg/m² to about 50 mg/m² more preferred in a dose of about 1 mg/m² to about 10 mg/m² and even more preferred at about 4 mg/m² to about 8 mg/m².

In a further embodiment vincristin is administered at a dose of about 0.1 mg/m² to 10 mg/m² more preferred in a dose of about 0.5 mg/m² to about 5 mg/m² and more preferred at about 0.8 mg/m² to about 2 mg/m² about once a week whereas the neurotoxicity is the dosage limiting factor. Most commonly solution of vincristin sulfate from about 0.1 mg/mL to about 10 mg/mL are administered with single doses of about 0.1 mg/m² to about 50 mg/m² more preferred in a dose of about 0.5 mg/m² to about 10 mg/m² and even more preferred from about 1 mg/m² to about 5.0 mg/m².

Specific Indications

Glioma

In one embodiment a pharmaceutical composition for the treatment of glioma and/or anaplastic astrocytoma comprises a combination of at least one immunostimulator, more preferred an antagonist, even more preferred an antisense oligonucleotide of TGF-beta and most preferred, an antisense oligonucleotide identified in the sequence listing under Seq. Id. No. 1-127 and even more preferred the sequences with Seq. Id. No. 22-48 and at least one antineoplastic chemotherapeutic agent selected from the group of BCNU, CCNU, liposomal pegylated telozolomid, procarbazine and vincristin.

In another embodiment the antineoplastic chemotherapeutic agents procarbazine, CCNU and vincristin are together with the immunostimulator, more preferred an antagonist, even more preferred an antisense oligonucleotide of TGF-beta and most preferred, an antisense oligonucleotide identified in the sequence listing under Seq. Id. No. 1-127 and even more preferred the sequence with Seq. Id. No. 22-48 are the components of a pharmaceutical com-

position. The dosage in this embodiment is about 40 mg/m² to about 80 mg/m² of procarbazine p.o. (days about 8 to about 21), about 80 to about 120 mg/m² CCNU, p.o. (about day 1), vincristin from about 1.2 mg/m² to about 1.8 mg/m² p.o. (day 1) with a maximum of about 2 mg/m² i.v. on about day 8, and about day 29. The immunostimulator is given before, with or after the administration of these three substances.

In another embodiment this cycle is repeated after about 6 to about 8 weeks once or several times.

In a further preferred embodiment the at least one immunostimulator more preferred an antagonist, even more preferred an antisense oligonucleotide of TGF-beta and most preferred, an antisense oligonucleotide identified in the sequence listing under Seq. Id. No. 1-127 and even more preferred the sequences with Seq. Id. No. 22-48 and temozolomid are the parts of a pharmaceutical composition. In this case the dosage of temozolomid for the treatment of unwanted neoplasms more preferred glioma and/or anaplastic astrocytoma is from about 120 to about 180 mg/m², p.o. on day 1 to 5 of a cycle. In a more preferred embodiment the immunostimulator is administered from about 1 µg/kg/day to about 50 mg/kg/day. The cycle is repeated about all 3 to 5 weeks.

In a more preferred embodiment of the above mentioned embodiments for the treatment of neoplasms such as glioma and/or anaplastic astrocytoma the immunostimulator is an antagonist of TGF-beta yet more preferred an antisense oligonucleotide identified in the sequence listing under Seq. Id. No. 1-127 and most preferred the oligonucleotides identified with the Seq. Id. No. 22 to 48.

In a further preferred embodiment for the treatment of glioma radiation is further administered according to standard schedules as described above. In one embodiment the radiation is applied together with the administration of the combination as described above. In other embodiments the radiation is applied before or after the administration of the pharmaceutical compositions

according to this invention.

Pancreas:

In one embodiment of pharmaceutical compositions for the treatment of neoplasms; more preferred pancreatic neoplasms at least one substance inhibiting cell proliferation and/or inducing cell death is selected from the group of cisplatin, carboplatin, cyclophosphamid, docetaxel, PEG-liposomal doxorubicin, etoposid, folin acid, 5-fluorouracil, mitoxantron paclitaxel, topotecan and/or treosulfan.

In more preferred embodiments for the treatment of neoplasms the antineoplastic chemotherapeutic agents paclitaxel or carboplatin are the at least one part of a pharmaceutical composition according to this invention. Paclitaxel from about 100 mg/m² to about 200 mg/m² more preferred about 175 mg/m² or carboplatin administered i.v. on day 1 of a cycle. This cycle is repeated after about 20 to about 30 days.

In yet another embodiment for the treatment of neoplasms such as pancreatic neoplasms the at least one antineoplastic chemotherapeutic agent of a pharmaceutical composition according to this invention is gemcitabine. Gemcitabine is administered in dosages of about 800 mg/m² to about 1200 mg/m², more preferred about 1000 mg/m² iv. Within about 10 min to about 60 min, more preferred within about 12 min to about 20 min. This application is repeated for about 5 to about 10 days.

In yet other embodiments paclitaxel together with carboplatin, docetaxel together with carboplatin, carboplatin together with cyclophosphamid, cisplatin together with treosulfan, etoposid, mitoxantron together with folin acid and 5-fluorouracil, topotecan, or PEG-liposomal doxorubicin are the at least one antineoplastic chemotherapeutic agent of a pharmaceutical composition for the treatment of pancreatic neoplasms.

In a more preferred embodiment of the above mentioned embodiments for the treatment of pancreatic neoplasm the antagonist is an antagonist of TGF-beta

yet more preferred an antisense oligonucleotide identified in the sequence listing under Seq. Id. No. 1-127 and even more preferred the sequence with Seq. Id. No. 22-48.

5 In another embodiment further to the administration of these pharmaceutical compositions, radiotherapy is applied according to standard schedules as described above.

Non small cell lung carcinoma (NSCLC)

10 IN one embodiment of a pharmaceutical composition for the treatment of nonn small cell lung carcinoma (NSCLC) the at least one antineoplastic chemotherapeutic agent is selected from the group of cisplatin, etoposid, carboplatin, mitomycin, paclitaxel, gemcitabine and vinorelbin.

In yet another embodiment for the treatment of NSCLC further radiation is applied according to schedules as described above.

15 In a further preferred embodiment cisplatin together with etoposid are the at least one antineoplastic chemotherapeutic agent being administered for the treatment of neoplasms such as NSCLC. IN amore preferred embodiment Cisplatine is administered with a dosage of about 40 mg/m² to about 80 mg/m² more preferred about 60 mg/m² is infused on about day 1 and etoposid with a dosage of about 80 mg/m² to about 150 mg/m² is infused within about 30 min
20 to about 200 min on days 1 to 3 of a cycle. In a further preferred embodiment additionally radiation of the lung takes place with about 1 Gy to about 2 Gy, about 1 to about 2 times per day with a complete dosage of about 30 Gy to about 60 Gy within one cycle. The radiotherapy is before parallel or after the administration of the pharmaceutical composition according to this invention. In
25 another preferred embodiment one cycle of this therapy comprises about 15 to about 30 days, more preferred about 22 days. About 1 to about 10 are applied.

In yet another embodiment for the treatment of NSCLC the at least one substance inhibiting the cell growth cisplatin (dosage of about 20 mg/m² to about

40 mg/m²) is infused during about 1 h on about days 1, 8, 29, 36 of one cycle or cisplatin (dosages of about 4 mg/m² to about 8 mg/m²) is infused each day of a cycle. In a further preferred embodiment radion is applied with a dosage of about 2 Gy and concomitant boost of about 0,5 Gy each day with a concomitant boost of about 0.3 to about 0.8 Gy per day and a maximum total dosage within one cycle of about 40 Gy to about 80 Gy, more preferred from about 50 Gy to about 70 Gy. The cycle has a length of about 25 to about 50 days, more preferred from about 30 to about 40 days, most preferred from about 32 to about 38 days.

In a more preferred embodiment of the above mentioned embodiments for the treatment of NSCLC the immunostimulator is an antagonist of TGF-beta yet more preferred an antisense oligonucleotide identified in the sequence listing under Seq. Id. No. 1-127 and even more preferred the sequences with Seq. Id. No. 1 to 21 are administered according to schedules as described above.

Gastrointestinal neoplasms

In another embodiment of this invention for the treatment of neoplasms, more preferred gastrointestinal neoplasms such as neoplasms of colon, stomach, liver and/or oesophagus the at least one antineoplastic chemotherapeutic agent is selected from the group of capecitabin, cisplatin, epirubicin, 5-fluorouracil, folin acid, Irinotecan, mitomycin C, oxaliplatin and taxotere.

In yet another embodiment for the treatment neoplasms such as oesophageal neoplasms 5-fluorouracil and cisplatin are the two antineoplastic chemotherapeutic agents of a pharmaceutical composition invention inhibiting cell proliferation. 5-fluorouracil with a dosage from about 800 mg/m² to about 1200 mg/m² is infused continuously, more preferred from day 1 to about day 5 of one cycle. Additionally cisplatin in a dosage from about 60 mg/m² to about 90 mg/m² is administered i.v., preferred on about day 1 of this cycle.

In even more preferred embodiments of the above mentioned embodiments for the treatment of gastrointestinal neoplasms the antagonist is an antagonist

of TGF-beta yet more preferred an antisense oligonucleotide identified in the sequence listing under Seq. Id. No. 1-127 and more preferred the sequences with Seq. Id. No. 1 to 21.

~~In a further preferred embodiment for the treatment of neoplasms such as~~

5 gastrointestinal neoplasms radiation is additionally applied with a total dosage of about 40 Gy to about 60 Gy within one cycle. Even more preferred this dosage is fractioned into about 5 times about 1 Gy to about 2 Gy per week. The cycle is repeated after about 20 to about 40 days, after about 25 to about 35 days or after about 30 days.

10 Melanoma

Further preferred embodiments are pharmaceutical compositions according to this invention for the treatment of neoplasms such as melanomas, wherein the at least one substance inhibiting cell proliferation and/or inducing cell death is selected from the group of BCNU, cisplatin, dacarbazin, DTIC, fotemustin,
15 interferon alpha, interleukin-2, interferone-alpha-2-a, temozolomid, vinblastin.

In even more preferred embodiments of the above mentioned embodiments for the treatment of melanoma the immunostimulator is an antagonist of TGF-beta yet more preferred an TGF-beta antisense oligonucleotide identified in the sequence listing under Seq. Id. No. 1-127 and even more preferred the se-
20 quence with Seq. Id. No. 1 to 78.

Prostate neoplasms

Further preferred embodiments are pharmaceutical compositions according to this invention for the treatment of neoplasms such as prostate neoplasms. In a preferred embodiment the at least one substance inhibiting cell proliferation
25 and/or inducing cell death is selected from the group of docetaxel, estramustinphosphate and mitoxantron.

In even more preferred embodiments of the above mentioned embodiments for the treatment of melanoma the antagonist is an antagonist of TGF-beta yet

more preferred an antisense oligonucleotide identified in the sequence listing under Seq. Id. No. 1-127 and even more preferred the sequences with Seq. Id. No. 1-21.

Pharmaceutical Technology

5 The pharmaceutical composition of an antagonist of this invention is delivered solely or in mixtures with the at least one substance inhibiting cell proliferation and/or inducing cell death. A mixture may consist of several antineoplastic agents in addition to immunostimulators, more preferred antagonists of factors negatively influencing the immune system, more preferred TGF-beta antago-
10 nists even more preferred TGF beta antisense oligonucleotides. These at least two substances herein is also referred to as compounds.

In one embodiment the at least two compounds are mixed and pure or in a pharmaceutical acceptable carrier. In yet another embodiment the at least two compounds of the pharmaceutical composition are separate and pure or are
15 separate and in a pharmaceutical acceptable carrier. In one embodiment the at least two components are in the same pharmaceutical acceptable carrier, in yet another embodiment the at least two components are in different pharmaceutical acceptable carriers.

Forms of administration

20 "Administering" the pharmaceutical compositions of the present invention may be accomplished by any means known to the person skilled in the art. Routes of administration include but are not limited to oral, intranasal, intratracheal, ocular, pulmonary, vaginal, rectal, parenteral (e.g. intramuscular, intradermal, intravenous, intratumoral or subcutaneous or direct injection), topical, trans-
25 dermal.

In one embodiment of a pharmaceutical composition for the treatment of unwanted neoplasms, the combination of at least one substance inhibiting cell proliferation and/or inducing cell death and the at least one immunostimulator are delivered by means of a biodegradable, polymeric implant or implanted

catheters.

The term "pharmaceutical composition" implicates the liquids or substances of this composition are pure and/or combined with pharmaceutical acceptable carriers.

- 5 The term "pharmaceutically-acceptable carrier" means one or more compatible solid or liquid filler, diluents or encapsulating substances which are suitable for administration to a human or other vertebrate animal. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of
- 10 the pharmaceutical compositions also are capable of being commingled with the compounds of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficiency.

Such carriers enable the compounds of the invention to be formulated as tablets, coated tablets, granules, powders, pills, dragees, (micro)capsules, liquids, gels, syrups, slurries, suspensions, emulsions and the like, for oral ingestion by a subject to be treated.

The pharmaceutical compositions may also include granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, creams, drops, coated onto microscopic gold particles or preparations with protracted release of active compounds, in whose preparation excipients and additives and/or auxiliaries such as disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners or solubilizers are customarily used as described above.

- 25 For a brief review of present methods for drug delivery, see Langer 1990, which is incorporated herein by reference.

Orale Forms

For oral administration, the compounds (i.e., at least one immunostimulator

and at least one substance inhibiting cell proliferation and/or inducing cell death) are delivered alone without any pharmaceutical carriers or formulated readily by combining the compound(s) with pharmaceutically acceptable carriers.

5 In one embodiment pharmaceutical preparations for oral use are obtained as solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations
10 such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP).

In yet another embodiment disintegrating agents are added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as
15 sodium alginate. Optionally the oral formulations may also be formulated in saline or buffers for neutralizing internal acid conditions.

In yet another embodiment dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethyl-
20 ene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures.

In yet another embodiment dyestuffs or pigments are added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

25 In another embodiment pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. In one embodiment the push-fit capsules contains the active ingredient in a mixture with filler such as lactose, binders such as starches, and/or lubricants such as talc

or magnesium stearate and, optionally, stabilizers. In another embodiment of the soft capsules, the active compounds are dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added.

- 5 In yet another embodiment microspheres formulated for oral administration are used, well known to someone skilled in the art.

The formulations for oral administration are in dosages suitable for such administration.

- 10 In yet another embodiment for buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

Inhalation

- 15 In yet another embodiment for the administration by inhalation, the compounds for use according to the present invention may be conveniently delivered in the form of an aerosol spray, from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g. gelatin for use in an inhaler or insufflator may be formulated containing a
20 powder mix of the compound and a suitable powder base such as lactose or starch.

Suitable pharmaceutical carriers are, for example, aqueous or saline solutions for inhalation, microencapsulated, emulsified, contained in liposomes, nebulized, aerosols.

25 Parenteral Application

In yet another embodiment the pharmaceutical acceptable carriers of the compounds for parenteral, intrathecal, intraventricular or intratumoral admini-

stration include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

5 In yet another embodiment for the systemic delivery of the compounds they are in pharmaceutical carriers for parenteral administration by injection (e.g., by bolus injection or continuous infusion). Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The pharmaceutical compositions take such forms
10 as suspensions, solutions or emulsions in oily or aqueous vehicles, and contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

In one embodiment pharmaceutical carriers for parenteral administration include aqueous solutions of the active compounds in water-soluble form.

IN yet another embodiment a suspensions of the compounds is prepared as
15 appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions comprise substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspen-
20 sion may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

In yet another embodiment the active compounds may are in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use
25 or dried onto a sharp object to be scratched into the skin.

Rectal, vaginal composition

In yet another embodiment the compounds are formulated in rectal or vaginal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

Depot-form

In yet another embodiment the compounds are formulated as a depot preparation. In one embodiment such long acting formulations are formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

In other embodiments delivery systems include time-release, delayed release or sustained release delivery systems. Such systems can avoid repeated administrations of the compounds, increasing convenience to the subject and the physician. Many types of release delivery systems are available and known to those of ordinary skill in the art.

In one embodiment the delivery system includes polymer base systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Pat. No. 5,075,109.

In another embodiment the delivery systems include non-polymer systems that are e.g. lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono-di-and tri-glycerides; hydrogel release systems; sytastic systems; peptide based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like.

Specific examples include, but are not limited to: (a) erosional systems in which an agent of the invention is contained in a form within a matrix such as those described in U.S. Pat. No. 4,452,775; 4,675,189, and 5,736,152, and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Pat. No. 3,854,480, 5,133,974 and 5,407,686. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

In still other embodiments, the antagonist and antineoplastic agent are formulated with GELFOAM, a commercial product consisting of modified collagen fibers that degrade slowly.

Gel-producer

5 In one embodiment the pharmaceutical compositions also comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

10 Salt derivatives

In one embodiment the immunostimulators and substances inhibiting cell proliferation and/or inducing cell death are administered neat or in the form of a pharmaceutically acceptable salt. The salts have to be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically acceptable salts thereof. Such salts include, but
15 are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, acetic, salicylic, p-toluene sulphonic, tartaric, citric, methane sulphonic, formic, malonic, succinic, naphthalene-2-sulphonic, and benzene sulphonic. Also, such salts can be prepared
20 as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts of the carboxylic acid group.

Buffers

IN one embodiment suitable buffering agents include but are not limited to: acetic acid and a salt (1-2% w/v); citric acid and a salt (1-3% w/v); boric acid
25 and a salt (0.5-2.5% w/v); and phosphoric acid and a salt (0.8-2% w/v).

Suitable preservatives include benzalkonium chloride (0.003-0.03% w/v); chlorobutanol (0.3-0.9% w/v); parabens (0.01-0.25% w/v) and thimerosal

(0.004-0.02% w/v).

Topic administration

In one embodiment the pharmaceutical acceptable carrier for topical administration for the at least two compounds of a pharmaceutical composition according to this invention include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. In yet another embodiment coated condoms, gloves and the like are useful.

- 10 In yet another embodiment the pharmaceutical compositions also include penetration enhancers in order to enhance the alimentary delivery. Penetration enhancers may be classified as belonging to one of five broad categories, i.e., fatty acids, bile salts, chelating agents, surfactants and non-surfactants (Lee et al. 1991, Muranishi 1990). One or more penetration enhancers from one or
15 more of these broad categories may be included.

Various fatty acids and their derivatives which act as penetration enhancers include, for example, oleic acid, lauric acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprinate, tricaprinate, ricinoleate, monoolein (a.k.a. 1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, mono- and di-glycerides and physiologically acceptable salts thereof (i.e., oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, etc.) (Lee et al. 1991, Muranishi, 1990, El-Hariri et al. 1992). Examples of some presently preferred fatty acids are sodium caprate and sodium
20 laurate, used singly or in combination at concentrations of 0.5 to 5%.

The physiological roles of bile include the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (Brunton, 1996). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus, the term "bile salt" includes any of the naturally occurring components of bile as

well as any of their synthetic derivatives. A presently preferred bile salt is chenodeoxycholic acid (CDCA) (Sigma Chemical Company, St. Louis, Mo.), generally used at concentrations of 0.5 to 2%.

Complex formulations comprising one or more penetration enhancers may be used. For example, bile salts may be used in combination with fatty acids to make complex formulations. Preferred combinations include CDCA combined with sodium caprate or sodium laurate (generally 0.5 to 5%).

Chelating agents

In one embodiment additionally chelating agents are used they include, but are not limited to, disodium ethylenediaminetetraacetate (EDTA), citric acid, salicylates (e.g., sodium salicylate, 5-methoxysalicylate and homovanilate), N-acyl derivatives of collagen, laureth-9 and N-amino acyl derivatives of beta-diketones (enamines) (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, 8:2, 92-192; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7:1, 1-33; Buur et al. 1990). Chelating agents have the added advantage of also serving as DNase inhibitors.

Surfactants

In yet another embodiment additionally surfactants are used. Surfactants include, for example, sodium lauryl sulfate, polyoxyethylene-9-lauryl ether and polyoxyethylene-20-cetyl ether (Lee et al. 1991); and perfluorochemical emulsions, such as FC-43 (Takahashi et al., 1988).

Non-surfactants include, for example, unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacyclo-alkanone derivatives (Lee et al. 1991); and non-steroidal anti-inflammatory agents such as diclofenac sodium, indomethacin and phenylbutazone (Yamashita et al. 1987).

Adjuvants

In one embodiment the pharmaceutical compositions of the present invention

additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions may contain additional compatible pharmaceutically-active materials such as, e.g., antipruritics, astringents, local anesthetics or
5 anti-inflammatory agents, or may contain additional materials useful in physically formulating various dosage forms of the composition of present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the invention.
10

EXAMPLES

Clinical studies represented herein were primarily designed as safety studies and were approved by the local ethic committees and performed in accordance with the current international declaration of Helsinki for human experimentation and GCP.
15

The treatment with the antineoplastic agent followed routine schedules if nothing else is mentioned. Before the treatment with an TGF-beta antagonist, the antisense oligonucleotide of TGF-beta, with Seq. No. 30 the patients were selected according to the following criteria:

20 Patients had AA, WHO grade III, or GBM, WHO grade IV, refractory to or recurrent after standard therapy (surgery, radiotherapy and different therapies with antineoplastic substances). Patients had not received antineoplastic agents within 10 days prior to the administration of the antagonist. Patients were between 18 and 75 years old. Karnofsky performance status (KPS) was
25 at least 70%. Patients with clinically significant acute infections, cardiovascular abnormalities or poorly controlled seizures and pregnant and lactating females were excluded.

Surgical planning was based on computer tomography or magnetic resonance images. The perforated part of the catheter was placed in the solid, enhancing

area of the tumor. Ventricles, cysts, resection cavities from prior surgical interventions, blood vessels and eloquent brain areas had to be avoided by the catheter trajectory. The catheter was introduced through a standard burr hole into the center of the largest tumor lesion. The distal end of the catheter was passed several centimetres under the galea through the skin and filled with saline. TGF-beta 2 specific antisense oligonucleotide with Seq. Id. No. 30 was administered intratumorally as a continuous high-flow microperfusion using an external pump system, Graseby 3200 (Smith Medical, London, GBM). The application system was removed after the end of the infusion. For safety assessment patients were followed up for 28 days. Post-study MRI and survival data until death were collected by the investigators.

1. Example

47 years old male who was diagnosed with a histologically grade III anaplastic astrocytoma received a combination therapy of several antineoplastic agents and TGF-beta 2 specific antisense oligonucleotide with Seq. Id. No. 30. The antineoplastic agents administered were ACNU together with tenoposide, temozolomide, and PEG-ylated liposomal doxorubicin (Caelyx®). ACNU was administered partly parallel with tenoposide with 90 mg/m² ACNU on the first day of each cycle and 60 mg/m² of tenoposide on days 1-3 of each cycle. Each cycle comprised 42 days, 4 of these cycles were realized. About 2 years later the patient was treated with 3 cycles of temozolomide. Each cycle of 28 days started with the administration of temozolomide 75 mg/m² from day 1-5. About 8 month after this treatment PEG-ylated liposomal doxorubicin (Caelix®) was administered in 5 cycles of 42 days, with 20 mg/m² on day 4 and day 14 of the, followed by a week with 160 mg tamoxifen administration in the morning and in the evening.

The therapy with these antineoplastic agents according to standard schedules was finally without success and therefore the patient was included into the study with TGF-beta 2 specific antisense oligonucleotide with Seq. Id. No. 30 showing surprising success. At the startpoint of this study the magnetic reso-

nance imaging showed three tumors in the left frontal lobe and an additional tumor in the right hemisphere and an overall edema. After the chemotherapy with the above mentioned antineoplastic agents one cycle of TGF-beta 2 specific antisense oligonucleotide with Seq. Id. No. 30 (10 μ M in steril pyrogen

5 free isotonic 0.9% NaCl solution, 4 μ L/min, total of 1.42 mg in 4 days) was applied intratumorally by an implanted catheter into the largest nodule. Six month after start of TGF-beta 2 specific antisense oligonucleotide with Seq. Id. No. 30 a clear reduction of the largest tumor lesion could be diagnosed. Although not individually targeted by the catheter, the three smaller tumors also
10 disappeared completely. Additionally, the edema had decreased. 17 months after the first application of TGF-beta 2 specific antisense oligonucleotide with Seq. Id. No. 30 the largest tumor was hardly measurable anymore. Four months later a complete response was assessed by 3 independent specialists. These findings were accompanied by clinical improvement. The patient died
15 due to a myocardial infarction without signs of tumor recurrence and had experienced an overall survival of 195 weeks after first recurrence and 208 weeks after diagnosis of anaplastic astrocytoma.

2. Example

Male patient about 45 years old was diagnosed with anaplastic astrocytoma
20 (WHO grade III). The diagnosis was followed by surgery and radiotherapy. 3 times 200 mg/m² Temozolomide was administered according to a standard schedule during two month. Again this therapy was without success. Therefore the patient was included into the study with TGF-beta 2 specific antisense oligonucleotide with Seq. Id. No. 30. Two cycles of this oligonucleotide with a
25 concentration of 80 μ M and a flow of 8 μ L/min was administered for 4 days through a catheter placed inside the tumor tissue. Afterwards the patient received ten additional of these cycles within four months. Following the last cycle of the oligonucleotide, approximately 10 months after the first oligonucleotide treatment, the patient received seven cycles of liposomal doxorubicin
30 (Caelyx®).

A planned 8th cycle could not be started, as the chemotherapy had to be discontinued due to cardiotoxicity (ventricular tachycardia). From that time the patient did not receive any anti-tumor therapy or corticosteroids. The last magnetic resonance image was taken 19.4 months after the start of oligonucleotide treatment. These images were evaluated and showed in the central reading a significant partial response (83% tumor reduction) and an overall survival time which was not so far reported in literatur.

This is a further prove, that surprisingly the coadministration of radiotherapy, antineoplastic agents and antagonists clearly show synergistic effects in the treatment of tumors, such as e.g. glioma.

3. Example

Comparison of survival data of patients treated with antineoplastic agents in combination with antagonists of factors negatively influencing the immune system (here: an antisense oligonucleotide of TGF-beta with the sequence Id. No. 30) to literature data for treatment with antineoplastic alone. Survival time is given from start of first chemotherapy after tumor recurrence. Median overall survival time of all patients treated with antineoplastic agents and TGF-beta 2 specific antisense oligonucleotide with Seq. Id. No. 30 (anaplastic astrocytom: 8 patients, glioblastoma, 23 patients) are compared to the most current literature data (Theodosopoulos, P.V. et al. 2001).

Table 1: Demographic data and patients' characteristics

Patient	Histology ¹ Steroid ⁸	Age (years)	Sex ²	KPS ³ at baseline	Tumor size ⁴ volume (cm ³)	Previous therapy ⁶ (after 1st recurrence)	Seq. Id. No. 30 (study group) ⁷ (cycles)	
01	AA	41	M	70	204.0	TMZ	1/1; 2/1 2x	40 mg MP
02	AA	46	M	90	216.0	Surg + TMZ, CaeTam	1/1 1x	-
03	GBM	61	M	70	73.5	CaeTam + Surg	1/1 1x	8 mg MP
04	AA	46	M	80	11.5 ⁵	Surg + TMZ	1/2 1x	12 mg DEX
06	GBM	51	F	70	76.8	Surg + TMZ	1/2 1x	-
07	GBM	53	M	70	54.9	Surg + TMZ, Surg + Ixotene	1/2 1x	24 mg DEX
08	GBM	56	M	70	101.0	TMZ	1/2 1x	2 mg DEX
09	GBM	63	F	70	67.7 ⁵	-	1/3 1x	3 mg DEX
10	GBM	30	M	90	160.7	Surg + TMZ, CaeTam	1/3 1x	12-8 mg DEX
11	GBM	43	M	70	16.7 ⁵	TMZ	1/3 1x	2 mg DEX
12	GBM	58	M	70	n/e	Surg + TMZ	1/3 1x	6 mg DEX
13	GBM	58	F	90	47.1 ⁵	-	1/4 1x	-
14	AA	54	M	80	7.2	Surg + TMZ	1/4 1x	-
15	GBM	42	M	70	33.6	Surg + TMZ, 2x Surg	1/4 1x	-
16	GBM	45	M	90	27.0	-	1/4 1x	-
17	AA	44	M	100	6.2 ⁵	Surg	1/5; 2/2 2x	-
18	GBM	46	M	70	n/e	TMZ + Surg	1/5 1x	-
19	GBM	41	F	70	58.8	Surg + TMZ	1/5 1x	-
Median		46		70	56.85			

¹ AA, anaplastic astrocytoma; GBM glioblastoma multiforme

² F, female; M, male

³ KPS, Karnofsky performance status

⁴ Tumor size at baseline

5 ⁵ Multiple lesions, the total volume of all lesions is presented

⁶ TMZ, temozolomide; CaeTam, Caelyx Tamoxifen; Surg, surgery

⁷ For details see Table 1

⁸ DEX, dexamethasone; MP, methylprednisolone

10 Summary of patients' characteristics from the study. Patients 01, 13 and 16 received each two cycles of pegylated liposomal doxorubicin (Caelyx®), patient 14 two cycles of PCV (procarbazine, lomustine (CCNU), vincristine) after TGF-beta 2 specific antisense oligonucleotide with Seq. Id. No. 30 treatment, all other patients had no anti-tumor therapy after oligonucleotide treatment.
15 Patient 17 received 10 additional oligonucleotide cycles. After the last cycle of the oligonucleotide the patient received 7 cycles of pegylated liposomal doxorubicin.

Reduction of tumor volumes of patients 04 and 017 were more than 80%. Tumor volume was assessed by measurement of the largest cross-sectional
20 diameter of the enhancing lesion in the first layer and the largest cross-sectional diameter perpendicular to the first in the same plane and layer. For the third dimension, the largest cross-sectional diameter of all further planes perpendicular to the first one was determined.

The median overall survival from the time of recurrence for anaplastic astrocytoma (AA) or glioma GBM (glioblastoma WHO grade IV) patients was about
25 93 weeks and about 44.0 weeks, respectively. Compared to literature data for the treatment with antineoplastic agents alone the survival data show clearly enhanced survival of patients treated with one or more antineoplastic agents (e.g. temozolomide and/or procarbazine before) before the administration of
30 TGF-beta 2 specific antisense oligonucleotide with Seq. Id. No. 30.

The data are calculated after start of chemotherapy. According to this approach the median overall survival in our study was 90.0 for AA and 42.4 for GBM. To allow direct comparison of our data to data of most current literature

we evaluated the patients that had received temozolomide prior to the oligonucleotide for the survival times after start of temozolomide therapy. Our data reveal longer median overall survival times if applying the oligonucleotide following temozolomide than the comparable published data for temozolomide alone: about 147 weeks versus 42 (Theodosopoulos, P.V. et al. 2001) weeks for AA, and 45 weeks versus about 32 weeks (Theodosopoulos, P.V. et al. 2001; Yung, W.K. et al. 2000; Yung, W.K. 2000; Brandes, A.A. et al. 2001) for GBM, respectively.

These results surprisingly show that there is a clear survival advantage of patients treated with a combination of an antagonist, e.g. TGF-beta 2 specific antisense oligonucleotide with Seq. Id. No. 30 and at least one further antineoplastic agent (e.g. temozolomide) in patients suffering from neoplasm, e.g. AA (mean overall survival of 146.6 weeks versus 90 weeks for all AA patients).

Example 4

Temozolomide may be administered orally in capsule form wherein it is admixed with conventional pharmaceutical carriers. An example for a temozolomide capsule formulation is:

Ingredient	mg/capsule			
	5	20	100	250
Temozolomide	5	20	100	250
Anhydrous Lactose NF	132.8	182.2	175.7	154.3
Sodium Starch Glycolate NF	7.5	11.0	15.0	22.5
Colloidal Silicon Dioxide NF	0.2	0.2	0.3	0.7
Tartaric Acid NF	1.5	2.2	3.0	9.0
Steric Acid NF	3.0	4.4	6.0	13.5
Capsule Size*	3	2	1	0

*) white opaque, preservativefree, twopiece hard gelatin capsules

The TGF-beta2 antisense oligonucleotide identified by the Seq. No. 30 is solved under sterile conditions in a sterile, pyrogenfree 0.9% NaCl solution and is ready for the administration into a catheter surgically implanted with its perforated end placed in the tumor. The catheter is connected with a commercially available port system into which the Ap12009 solution is administered.

Example 6

Antisense m-RNA for the human transforming growth factor TGF-beta 1:

```
CTGCAGCCTTGACCTCCAGGATCAAGTGATCCTCCACCTTAGCCTCCAGAGTAGCTGGGACCACAGGTGTA
10 CATTTTTTAAAAGTGTTTTGTAGAGATAGGGTCTCACTATGTTACCCAGGCTGGTCTCAAATGCCTGGATTCA
AGTATCCTCCCATCTCTGCCTCCCAAAGTGCTAGGATTACAGGCGTGAGACCCCGCCTGGCCTGAACCTACT
ATCTTTTATTGTCTTCTTCACTATCCCCACTAAAGCAGGTTCTTGGTGGGCAGGAACCTCCTCCCTTAACCTC
TCTGGGCTTGTTTTCTCAACCTTTAAAATGGGTGTTATCAGAGTCCCTGCCATCTCAGAGTGTGCTATGGTG
ACTGAATGAGTTCATTAATGTAAGGCACCTTCAACAGTGCCCAAGGTGCTCAATAAATAGATCTAACTACAGTA
15 GTGTTCCCACTGGTCCCTGTGCCTTGATGCCGGGCAAAGGAATAGTGCAGACAGGCAGGAGGAGGCAGAGA
GGGAGAGAGAGGGAGTGGGAGTGGGGGAACGTCAGGGATGGAGACCCAGGCAGGCGCCCAATGACACAGAGA
TCCGCAGTCCTCTCTCCATCTTTAATGGGGCCCCAGGTGGGCTTGGGGCACGGTGTCTTAAATACAGCCCCC
ATGGGCAAGGCAGCGGGGGCGGGGCGGGGTGGGGCCGGGCCTGCCGGGGCGGGGCGGGGCGGGGCGGGACCTC
AGCTGCACCTTGACAGAGCGACGATCATGTTGGACAGCTGCTCCACCTTGGGCTTGCGGCCACGTAGTACAC
20 GATGGGCAGCGGCTCCAGCGCCTGCCGCACGCAGCACGGCGCCGCCAGGCGCCCGGGTTATGCTGGTTGTAC
AGGGCCAGGACCTTGCTGTACTGCGTGTCCAGGCTCCAAATGTAGGGGCAGGGCCCCAGGCAGAAAGTTGGCAT
GGTAGCCCTTGGGCTCGTGGATCCACTTCCAGCCGAGGTCTTGCAGGAAGTCAATGTACAGCTGCCGCACGCA
GCAGTTCTTCTCCGTGGAGCTGAAGCAATAGTTGGTGTCCAGGGCTCGGCGGTGCCGGGAGCTTTGCAGATGC
TGGGCCCTCTCCAGCGGGGTGGCCATGAGAAGCAGGAAAGGCCGGTTCATGCCATGAATGGTGGCCAGGTAC
25 CTCGGCGGCCGGTAGTGAACCCGTTGATGTCCACTTGACAGTGTGTTATCCCTGCTGTACAGGAGCAGTGGGC
GCTAAGGCGAAAGCCCTCAATTTCCCTCCACGGCTCAACCACTGCCGCACAACTCCGGTGACATCAAAGAT
AACCCTCTGGCGAGTCGCTGGGTGCCAGCAGCCGGTGTCTGAGGTATCGCCAGGAATTGTTGCTGTATTTCT
GGTACAGCTCCACGTGCTGCTCCACTTTTAACTTGAGCCTCCTCAGCAGACGCAGCTCTGCCCGGGAGAGCAA
CACGGGTTACAGGTACCGCTTCTCGGAGCTCTGATGTGTTGAAGAACATATATATGCTGTGTGTACTCTGCTTG
30 AACTTGTCATAGATTTCTGTTGTGGGTTTCCACCATTAGCACGCGGGTGACCTCCTTGGCGTAGTAGTCGGCCT
CAGGCTCGGGCTCCGGTTCTGCACTCTCCCCGGCCACCCGGTCCGGGTGCTGTTGTACAGGGCGAGCACGGC
CTCGGGCAGCGGGCCGGGCGGCACCTCCCCCTGGCTCGGGGGGCTGGCGAGCCGCAGCTTGGACAGGATCTGG
CCGCGGATGGCCTCGATGCGTTCCGCTTACCAGCTCCATGTCGATAGTCTTGACAGGTGGATAGTCCCGCGG
CCGGCGGGCCAGGCGTCAGCACCAGTAGCCACAGCAGCGGTAGCAGCAGCGGCAGCAGCCGAGCCCGGAGGG
35 CGGCATGGGGGAGGCGGCGCCCCCGGCACTGCCGAGAGCGCGAACAGGGCTGGTGTGGTGGGGAGGCCCCGC
CCCTGCAGGGGTGGGGGTCTCCCGGCAAAGGTAGGAGGGCCTCGAGGGAAAGCTGAGGCTCCTCAGGGAGA
AGGGCGCAGTGGTGGAGGGGAGGCTTGGACCGGGGTGTCTCAGTATCCACGGAAATAACCTAGATGGGCGC
```

GATCTGGTACCAGAAGGTGGGTGGTCTTGAATAGGGGATCTGTGGCAGGTCCGAGAGAGATCCGTCTCCTGGA
GGAGAAAAGGTCTAGGATGCGCGGGGGCTCAGGAGACAGGCCGGGGATGAAGGCGGCGTGCAGGGGGTGCGCC
CGAGGTCTGGGGAAAAGTCTTTGCGGGAGGCCGGGTGCGCGACTCCCGAGGGCTGGTCCGGAATGGGGGCGCC
TGAGGGACGCCGTGTAGGGGGCAGGGAGGGAGCAAGCGTCCCCGGCGGCAAAGGGAGGCGGTCTGGGGTCCCC
5 AAGTCCTGCCTCCTCGCGGGGCAGGGTCGCGCCAAGAGGTCCCCGCGCCTCCGGCTCCCGAGGGCAACGGAAA
AGTCTCAAAAGTTTTTTTCTCTCTCCCGACCAGCTCGTCCCTCCTCCGCTCCTCCTCCCCCTCCTCCCCG
CAGTGGCGGGGGCGGCGGGCTCGTCTCAGACTCTGGGGCTCAGGCTGCTCCTCGGCGACTCCTTCCTCCG
CTCCGGGCGGAGGCCGGCCCCGCGGGCGGCTCAGAGCCGGGGGGGGTGCCCCGGACGGGGCGTCCCCCTGCC
CCCGGCCGGGGCCCTCGCTGTCTGGCTGCTCCGCGGAGGGAGGT

10 Antisense m-RNA of the human transforming growth factor TGF-beta2:

TTTAAAAAATTGCTTCTTGTCTCTCTCACTTACAAAGTAGGTGAAATGTAGAATAAGGCCTTCAACTTTTT
TTGTGTCAGATGCCAGTTTTTAACAAACAGAACACAAACTTCCAAAGTGCTGAACTAGTACCGCCTTTTCAA
AATTTTTTAACACTGATGAACCAAGGCTCTCTTATGTTTTCTTGTTACAAGCATCATCGTTGTGCTCGTCATC
15 ATCATTATCATCATCATTGTCAATTTGGTCTTGCCACTTTTCCAAGAATTTTAGCTGCATTTGCAAGACTTTA
CAATCATATTAGAAAGCTGTTCAATCTTGGGTGTTTTGCCAATGTAGTAGAGAATGGTTAGAGGTTCTAAATC
TTGGGACACGCAGCAAGGAGAAGCAGATGCTTCTGGATTATGGTATTATATAAGCTCAGGACCCTGCTGTGC
TGAGTGTCTGAACTCCATAAATACGGGCATGCTCCAGCACAGAAGTTGGCATTGTACCCCTTTGGGTTCTGTGA
TCCATTTCACCCTAGATCCCTCTTGAAATCAATGTAAAGTGGACGTAGGCAGCAATTATCCTGCACATTTCT
AAAGCAATAGGCCGCATCCAAAGCACGCTTCTCCGCGGTTGGTCTGTTGTGACTCAAGTCTGTAGGAGGGC
20 AATAACATTAGCAGGAGATGTGGGGTCTTCCCACTGTTTTTTTTCTAGTGGACTTTATAGTTTTCTGATCAC
CACTGGTATATGTGGAGGTGCCATCAATACCTGCAATCTTGCTTCTAGTTCTTCACTTTTATTTGGGATGAT
GTAATTATTAGATGGTACAAAGTGCAGCAGGGACAGTGTAAAGCTTATTTTAAATCCAGGTTCTGTCTTTA
TGGTGAAGCCATTATGAACAGCATCAGTTACATCGAAGGAGAGCCATTGCGCTTCTGCTCTTGTTTTCACAA
CTTTGCTGTGATGTAGCGCTGGGTGAGATGTTAAATCTTTGGACTTGAGAATCTGATATAGCTCAATCCG
25 TTGTTCAAGCACTCTGGCTTTTGGGTCTGCAACGAAAGACTCTGAACTCTGCTTTACCAAATTGGAAGCA
TTCTTCTCCATTGCTGAGACGTCAAATCGAACAATTCTGAAGTAGGGTCTGTAGAAAGTGGGCGGGATGGCAT
TTTCGGAGGGGAAGAAGGGCGGCATGTCTATTTTGTAACCTCCTTGGCGTAGTACTCTTCGTCGCTCCTCTC
GCGCTCGCAGGCGGCGCCCTCCGGCTCGCCTTCTCCTGGAGCAAGTCCCTGGTGTGTTGTAGATGGAAATC
ACCTCCGGGGGGACTTCTCGGGCTCAGGATAGTCTTCTGGGGGACTGGTGAGCTTCAGCTTGCTCAGGATCT
30 GCCCGCGGATCGCCTCGATCCTCTTGCGCATGAACTGGTCCATATCGAGTGTGCTGCAGGTAGACAGGCTGAG
CGCGACCGTGACCAGATGCAGGATCAGAAAAGCGCTCAGCACACAGTAGTGCAATTTTTTAAAAAGTGGAAAA
AAAAGTTGTTTTTAAAGTCAGAATAAAAAAAGAAATCAACAATCTCAAAGTATAGATCAAGGAGAGTTG
TTTGGTTTTTTGTTGTTGTTGTTGTTTTGATGCGAAACTTTTGCAAACAATCTAGTCAATGCCCAACAGAA
AAACGTATCCTGCTTG

35 Antisense of m-RNA of human TGF-beta

CAGGATGCCCCAAAAATATTTATTTATACAAAGATTTTGAGAGTAATATTCATACTTGTCTTTATACCTCAGT
CTATGCGTCTGGGGCCAAGTCACTGTGTGGCACATGTGAGCTTCCCCGAATGCCTCACATGTTGTGCGACCT
GCTTCCAGGAACACCAAATGAACACAGGGTCTTGGAGGGGAAGTGGGGGAAGAACCATAATGCCCAACCT
GCATGGAACCACAATCCAGAAATGTGCATCCTGACCTGGAAGGCGTCTAACCAAGTGTCCAAGGGGAAATATG
40 ATCGAGGGAGAGGTGAGAGGAGGGACCCAGAGGCAGACAGGAGAGGGTTGATTTCCACCCTTTCTTCTGCGTT

CAGCATATCCAAAAGGCCCAATACAGTTGATGGGCCAGGAACTGCATGACCTGGATTTTCTCCCTGTAGTGAC
CCACGATGTTAATTGATGTAGAGGACAGTTTGCAAAAGTAATAGATTTGCCCTTAATCCCAGACAGTATGAGA
TACAATTCTGGGACTTTGTCTTCGTAACCTGTCTTTAAAAAATAATGCTTGCCCTGTATAACATAAT
CCAGATTCCCTAGAGCAGATGTGGTACAGCAATGAGCAAATCCAACCTCAGATCTGAAGTGTCTCCAGTCTG
5 GCCCTGACCCAGCCATTCTCTGCCCTTCCTTCTCCCTTTAGGGTAGCCCAAATCCCATTGCCACACAACATCT
CAACTTACCATCCCTTTCTCTATCCCCATCCCCTCTGTCTGCGTCACAGAAAGTCTGTGTGTCTGAAGAGT
TCAGCCTTCTCTAACCACCCACACTTCTTTACCACCGTGATTCTCAGAGCCAGCAAGAAAGAAATGTTT
CAAAAGGAAACCTCCATCTCAGCCATTTGCCCGGAGCCGAAGGTTGTGGGCTCCAGGCCCTCAGTGAGGTTT
GTTGCTTGTGTGTTTCCCGAGGAGCGGGCAGTCAGGCAGTGGTGGTTCTCTCTCCCTCTCTCTGTCTCGCACGT
10 GGGGTCTCAGCTAATTTACAAGACTTCACCACCATGTTGGAGAGCTGCTCCACTTTGGGGGTCTCCCAACA
TAGTACAGGATGGTCAGGGGCTCCAGGTCTGGGGCAGCAGCAAGGCGAGGCAGATGCTTCAGGGTTCAGAG
TGTTGTACAGTCCCAGCACCGTGTGTGGGTTGTGTCTGCACTGCGGAGGTATGGGCAAGGGCTGAGCAGAA
GTTGGCATAGTAGCCCTTAGGTTTCATGGACCCACTTCAGCCCAGATCCTGTGCGAAGTCAATGTAGAGGGGG
CGCACACAGCAGTTCTCTCCAAGTTGCGGAAGCAGTAATTGGTGTCCAAAGCCCGCTTCTTCTCTGACCCC
15 CCTGGCCCCGGGTTGTGCGAGCCGGTGTGGGGGAATCATCATGAGGATTAGATGAGGGTTGTGGTGATCCTTCTG
CTTCTTGAGGCGCCCCAGATCTCCACGGCCATGGTCATCCTCATTGTCCACGCCCTTGAATTTGATTTCCATC
ACCTCGTGAATGTTTTCCAGGATATCTCCATTGGGCTGAAAGGTGTGACATGGACAGTGAATGCTGATTTCTA
GACCTAAGTTGGACTCTCTTCTCAACAGCCACTCACGCACAGTGTGAGTGACATCAAAGGACAGCCACTCGGC
AGTGCCCCGTGTGGGCAGATTCTTGCCACCGATATAGCGTGTGTTGGCAATGTGCTCATCTGGCCGAAGGATC
20 TGGAAGAGCTCGATCCTCTGCTCATTCCGCTTAGAGCTGGGGTTGGGCACCCGCAAGACCCGGAATTCTGCTC
GGAATAGGTTGGTCTATTTTCTCCACTGAGGACACATTGAAGCGGAAAACCTTGAGGTAATTCCTTTAGG
GCAGACAGCCAGTTCGTTGTGCTCCGCCAGCCCCCTGGATCATGTGCAATTTATGGATTTCTTTGGCATAGTAT
TCCGACTCGGTGTTTCTGGGTGCAGCCTTCTCCCTCTCCCCATGCATCTCCTCCAGCAGCTCCCGGGTGC
TGTTGTAAAGGGCCAGGACCTGATAGGGGACGTGGGTATCACCGTTGGCTCAGGGGGGCTGGTGAGCCTGAG
25 CTTGCTCAAGATCTGTCCCTAATGGCTTCACCCTCTTCTTCTTGATGTGGCCGAAGTCCAAGGTGGTGCAA
GTGGACAGAGAGAGGCTGACCGTGGCAAAGTTCAGCAGGGCCAGGACCACCAGAGCCCTTTGCAAGTGCATCT
TCATGTGTGAGCTGGGAAGAGAGGCCAGGGGGACGGCAAGGCCTGGAGAGGAAGAGACCCAGCAGACGTGCA
GAAGGAGGGAGGAAAACCAGGCGGCCTCCCAGATCCCAAAGACTGAGGCTTGGAAGAAGGTGCATGAACTC
ACTGCACTGCGAGAGCTTCAGGACTTCAGGAAGCGCTGGCAACCCTGAGGACGAAGAAGCGGACTGTGTGCC
30 TTGTAGCGCTGGGATTCTTGTCATGTGTCTAAACAGGTTTTGCTGG

Antisense of m-RNA of human Interleukine 10

TCACCCATATGGAACAGCTTAAAAACAGGTGAAAATAATAAATATTGAAAAAATTATAATATTGGGCTTCTT
TCTAAATCGTTCACAGAGAAGCTCAGTAAATAAATAGAAATGGGGTTGAGGTATCAGAGGTAATAAATATTC
TATAAGAGAGGTACAATAAGGTTTCTCAAGGGGCTGGGTGAGCTATCCCAGAGCCCCAGATCCGATTTTGGAG
35 ACCTCTAATTTATGTCCTAGAGTCTATAGAGTCGCCACCCTGATGTCTCAGTTTCGTATCTTCATTGTCTATG
AGGCTTCTATGTAGTTGATGAAGATGTCAAACCTCACTCATGGCTTTGTAGATGCCTTTCTCTTGAGCTTATT
AAAGGCATTCTTCACCTGCTCCACGGCCTTGCTCTTGTTTTACAGGGAAGAAATCGATGACAGCGCCGTAGC
CTCAGCCTGAGGGTCTTCAGGTTCTCCCCAGGGAGTTCACATGCGCCTTGATGTCTGGGTCTTGGTTCTCAG
CTTGGGGCATCACCTCCTCCAGGTAAAACTGGATCATCTCAGACAAGGCTTGGAACCCAGGTAACCCTTAA
40 GTCCTCCAGCAAGGACTCCTTTAACAACAAGTTGTCCAGCTGATCCTTCATTTGAAAGAAAGTCTTCACTCTG

CTGAAGGCATCTCGGAGATCTCGAAGCATGTTAGGCAGGTTGCCTGGGAAGTGGGTGCAGCTGTTCTCAGACT
GGGTGCCCTGGCCTGGGCTGGCCCTCACCCAGTCAGGAGGACCAGGCAACAGAGCAGTGCCTGAGCTGTGCAT
GCCTTCTTTTGCAAGTCTGTCTTGTGGTTTGGTTTTGCAAGAGCAACCCCTGATGTGTAGACCTTCACCTCT
CTGTCCCCCTTTTATATTGTAAGCTCAGGGAGGCCTCTTCATTCAATTAATAAAGCCACAATCAAGGTTTTCCCGG
CACAGGATTTTTCTGCTTAGAGCTCCTCCTTCTCTAACCTCTCTAATAAACTTAGTTTTCAATTTTTGCATC
GTAAGCAAAAATGATTGGTTGAACATGAACCTTCTGCATTACAGCTATTTTTAGGATGGGCTACCTCTCTTAGA
ATAATTTTTTAGCTTCTCAATTAATAAAGTTGATTCTCTGGGGAGAACAGCTGTTCTGTCCGCAGAGGCCCT
CAGCTGTGGGTTCTCATTGCGGTGTTCTTAGGTCACAGTGACGTGGACAAATTGCCCATTCAGAATACAATG
GGATTGAGAAATAATTGG

10 Antisense m-RNA of human Prostaglandin E₂ Synthase

[illegible]

Antisense m-RNA of human VEGF

CAGTGTGCTGGCGGCCGCGGTGTGTCTACAGGAATCCAGAAATAAACTCTCTAATCTTCCGGGCTCGGTGA
 TTTAGCAGCAAGAAAAATAAATGGCGAATCCAATTCCAAGAGGGACCGTGCTGGGTACCCGCCCGGGAATG
 CTTCCGCCGGAGTCTCGCCCTCCGGACCCAAAGTGCTCTGCGCAGAGTCTCCTCTTCCTTCATTTCAAGTTTC

TGGATTAAAGGACTGTTCTGTGCGATGGTGTGGTGGTGGCGGCAGCGTGGTTTCTGTATCGATCGTTCTGTA
TCAGTCTTTCCCTGGTGAGAGATCTGGTTCCCGAAACCCTGAGGGAGGCTCCTTCCTCCTGCCCGGCTCACCGC
CTCGGCTTGTACATCTGCAAGTACGTTTCGTTTAACTCAAGCTGCCTCGCCTTGCAACGCGAGTCTGTGTTTT
TGCAGGAACATTTACACGTCTGCGGATCTTGTACAAACAATGCTTTCTCCGCTCTGAGCAAGGCCCACAGGG
5 ATTTTCTTGTCTTGCTCTATCTTTCTTTGGTCTGCATTACATTTGTTGTGCTGTAGGAAGCTCATCTCTCCT
ATGTGCTGGCCTTGGTGAGGTTTGATCCGCATAATCTGCATGGTGATGTTGGACTCCTCAGTGGGCACACACT
CCAGGCCCTCGTCATTGCAGCAGCCCCCGCATCGCATCAGGGGCACACAGGATGGCTTGAAGATGTACTCGAT
CTCATCAGGGTACTCCTGGAAGATGTCCACCAGGGTCTCGATTGGATGGCAGTAGCTGCGCTGATAGACATCC
ATGAACCTCACCACCTTCGTGATGATTCTGCCCTCCTCCTTCTGCCATGGGTGCAGCCTGGGACCACTTGGCAT
10 GGTGGAGGTAGAGCAGCAAGGCGAGGCTCCAATGCACCCAAGACAGCAGAAAGTTCATGGTTTTCGGAGGCCCG
ACCGGGGCCGGGCCGGCTCGCGCCGGGCCGCCAGCACACTG

Example 7

Small molecules inhibiting TGF-beta

15 **SB-431542** TBRI kinase inhibitor from GlaxoSmithKline (Callahan et al. 2002, Laping et al. 2002; Inman et al. 2002)

NPC30345 TBRI kinase inhibitor from Scios, Inc. (Dumont & Arteaga 2003)

SD-093 TBR-I kinase inhibitor (Subramanian, G. et al. 2003).

LY364947 TBRI kinase inhibitor from Lilly Inc. (Sawyer et al. 2003).

20 **Decorin** a small chondroitin-dermatan sulfate proteoglycan that binds various forms of active TGF- β (Border et al. 1992).

Proteins inhibiting TGF-beta

Endoglin a TGF- β binding 95 kDa glycoprotein (Gougos et al. 1992).

Antibodies binding TGF-beta

25 **CAT-192** humanized TGF-beta1 mAB from Genzyme/CAT (Benigni et al. 2003).

CAT-152 humanized TGF-beta2 mAB from Genzyme/CAT (Siriwardena et al. 2002).

1D11 TGF-beta1, 2, 3 mAB from Genzyme/CAT (Ananth et al. 1999).

2G7 TGF-beta1, 2, 3 monoclonal IgG2 from Genentech., (Arteaga et al. 1993).

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systems, McKinley Place NE, Minneapolis , MN USA 55413

rabbit anti-TGF-beta2 LAP: (Schlotzer-Schrehardt, U. et al. 2001).

Soluble Receptors

-
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sTBRII:Fc (Yang, Y.A. et al. 2002)

Betaglycan (recombinant soluble TBRIII) (Bandyopadhyay et al. 2002)

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Sequenzen:

	No.	Sequences	Length	No. int.	Bez. int.
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	2	GTCGATAGTCTTGC	14	2	
	3	CTTGGACAGGATCT	14	3	
	4	CCAGGAATTGTTGC	14	4	
	5	CCTCAATTTCCCCT	14	5	
	6	GATGTCCACTTGCA	14	6	
	7	CTCCAAATGTAGGG	14	7	
	8	ACCTTGCTGTACTG	14	8	
	9	GTAGTACACGATGG	14	9	
	10	CACGTAGTACACGA	14	10	
	11	CATGTTGGACAGCT	14	11	
	12	GCACGATCATGTTG	14	12	
	13	TGTA CTCTGCTTGAAC	16	13	
	14	CTGATGTGTTGAAGAACA	18	14	
	15	CTCTGATGTGTTGAAG	16	15	
	16	GGAAGTCAATGTACAG	16	16	
	17	CATGTCGATAGTCTTGCA	18	17	
	18	AGCTGAAGCAATAGTTGG	18	18	
	19	GTCATAGATTTTCGTTGTG	18	19	
	20	CTCCACTTTTAACTTGAG	18	20	
TGF-beta 2	21	TGCTGTATTTCTGGTACA	18	21	
	22	CACACAGTAGTGCA	14	1	
	23	GCACACAGTAGTGC	14	2	
	24	GCTTGCTCAGGATCTGC	17	3	
	25	TACTCTTCGTCGCT	14	4	
	26	CTTGGCGTAGTACT	14	5	
	27	GTAAACCTCCTTGG	14	6	
	28	GTCTATTTTGTAACCTCC	19	7	
	29	GCATGTCTATTTTGTAACCC	20	8	
	30	CGGCATGTCTATTTTGTA	18	9	
	31	GGCATCAAGGTACC	14	10	
	32	CTGTAGAAAGTGGG	14	11	
	33	ACAATTCTGAAGTAGGGT	18	12	
	34	TCACCAAATTGGAAGCAT	18	13	
	35	GCTTTCACCAAATTGGAAGC	20	14	
	36	CTGGCTTTTGGGTT	14	15	
	37	TCTGATATAGCTCAATCC	18	16	
	38	TCCTAGTGGACTTTATAG	18	17	
	39	TTTTTCCTAGTGGACT	16	18	
	40	CAATTATCCTGCACATTTT	19	19	

	41	GCAATTATCCTGCACA	16	20	
	42	GCAGCAATTATCCTGC	16	21	
	43	TGGCATTGTACCCT	14	22	
	44	TGTGCTGAGTGTCT	14	23	
	45	CCTGCTGTGCTGAGTG	16	24	
	46	CTTGGGTGTTTTGC	14	25	
	47	TTTAGCTGCATTTGCAAG	18	26	
	48	GCCACTTTTCCAAG	14	27	
TGF-beta 3	49	TCGAGCTTCCCCCA	14	107	TGF-β3-98-1
	50	CCCCGAGCCCCAAGG	14	108	TGF-β3-98-2
	51	CCCGACGAGCCGG	13	109	TGF-β3-98-3
	52	ACGCACCAAGGCGA	14	110	TGF-β3-98-4
	53	CGGGTTGTCGAGCCC	15	111	TGF-β3-98-5
	54	CGGCAGTGCCCCG	13	112	TGF-β3-98-6
	55	CGCAATTCTGCTCG	14	113	TGF-β3-98-7
	56	TTCGTTGTGCTCC	14	114	TGF-β3-98-8
	57	ATTCCGACTCGGTG	14	115	TGF-β3-98-9
	58	ACGTGCGTCATCACCGT	17	116	TGF-β3-98-10
	59	CCAAGAAGCC	10	117	TGF-β3-98-11
	60	CCTAATGCCTTCCA	14	118	TGF-β3-312
	61	TCAGCAGGGCCAGG	14	187	GF-β-3rwk-1
	62	GCAAAGTTCAGCAGGGC	17	188	GF-β-3rwk-2
	63	GGCAAAGTTCAGCAGG	16	189	GF-β-3rwk-3
	64	GTGGCAAAGTTCAGCAGG	18	190	GF-β-3rwk-4
	65	GTGGCAAAGTTCAG	14	191	GF-β-3rwk-5
	66	GACCGTGGCAAAGTTCAG	18	192	GF-β-3rwk-6
	67	AGAGAGGCTGACCGT	15	193	GF-β-3rwk-7
	68	GAGAGAGAGAGGCTGAC	17	194	GF-β-3rwk-8
	69	ACAGAGAGAGGCTGA	15	195	GF-β-3rwk-9
	70	GTGGACAGAGAGAGG	15	196	GF-β-3rwk-10
	71	CAACTGGACAGAGAGAGG	18	197	GF-β-3rwk-11
	72	TCTTCTTGATGTGGCC	16	198	GF-β-3rwk-12
	73	CCCTCTTCTTCTTGATG	17	199	GF-β-3rwk-13
	74	CACCCTCTTCTTCT	14	200	GF-β-3rwk-14
	75	ATGGATTTCTTTGGCAT	17	201	GF-β-3rwk-15
	76	GGATTTCTTTGGC	13	202	GF-β-3rwk-16
	77	AAGTTGGACTCTCTTCTC	18	203	GF-β-3rwk-17
	78	TAAGTTGGACTCTCTTCT	18	204	GF-β-3rwk-18
PGE	79	TAGGAGTGGTTGAGGC	16	1539	Prostaglan.Rec.EP3-1
	80	GTGTAGGAGTGGTTGAG	17	1540	Prostaglan.Rec.EP3-2
	81	CTGTGTAGGAGTGG	14	1541	Prostaglan.Rec.EP3-3
	82	CCCACATGCCTGTG	14	1542	Prostaglan.Rec.EP3-4
	83	CGATGAACAACGAG	14	1543	Prostaglan.Rec.EP3-5

VEGF	84	CTGGCGATGAACAACG	16	1544	Prostaglan.Rec.EP3-6
	85	CGCTGGCGATGAAC	14	1545	Prostaglan.Rec.EP3-7
	86	GAGCTAGTCCCGTTG	15	1546	Prostaglan.Rec.EP3-8
	87	GCGAAGAGCTAGTCC	15	1547	Prostaglan.Rec.EP3-9
	88	CCAGTTATGCGAAGAGC	17	1548	Prostaglan.Rec.EP3-10
	89	CCCCAGTTATGCGAAG	16	1549	Prostaglan.Rec.EP3-11
	90	CGGCCGCGGTGTGT	14	119	VEGF-98-1
	91	CGGGAATGCTTCCGCCG	17	120	VEGF-98-2
	92	CGGCTCACCGCCTCGGC	17	121	VEGF-98-3
	93	CACGTCTGCGGATC	14	122	VEGF-98-4
	94	CCCCGCATCGCATCAGGG	18	123	VEGF-98-5
	95	CGCCTTGCAACGCG	14	124	VEGF-98-6
	96	CCGACCGGGGCCGG	14	125	VEGF-98-7
	97	GTTTCATGGTTTCGG	14	126	VEGF-49
	98	GCAGAAAGTTCATGG	15	127	VEGF-55
	99	GCTGATAGACATCC	14	128	VEGF-188
	100	GCGCTGATAGACAT	14	129	VEGF-190
	101	GTAGCTGCGCTGATAG	16	130	VEGF-194
	102	CTCGATCTCATCAG	14	131	VEGF-253
	103	ATGTACTCGATCTCATC	17	132	VEGF-255
	104	GAAGATGTACTCGATC	16	133	VEGF-260
	105	CTTGAAGATGTACTCG	16	134	VEGF-263
	106	GCATCGCATCAGGG	14	135	VEGF-292
	107	CCGCATCGCATCAG	14	136	VEGF-294
	108	CATTTGTTGTGCTGTAGG	18	137	VEGF-422
	109	GGTCTGCATTCACATTTG	18	138	VEGF-434
	110	CTTTGGTCTGCATTC	15	139	VEGF-441
	111	CTTTCTTTGGTCTGC	15	140	VEGF-445
	112	GCTCTATCTTTCTTTGG	17	141	VEGF-450
	113	GTCTTGCTCTATCTTTC	17	142	VEGF-455
	114	CTTGTCTTGCTCTATC	16	143	VEGF-459
	115	CATCTGCAAGTACGTTTCG	18	144	VEGF-596
	116	CACATCTGCAAGTACGTT	18	145	VEGF-598
	117	GTCACATCTGCAAGTACG	18	146	VEGF-600
	118	CATCTGCAAGTACG	14	147	VEGF-600-2
	119	CACATCTGCAAGTAC	15	148	VEGF-601
	120	GTCACATCTGCAAG	14	149	VEGF-604
	121	CTTGTCACATCTGC	14	150	VEGF-607
	122	GGCTTGTCACATCTGC	16	151	VEGF-607-2
	123	CTCGGCTTGTCACATC	16	152	VEGF-610
	124	CTCCTTCCTCCTGC	14	153	VEGF-638

IL-10	125 GCTTGAAGATGTACCTCG	16	154 VEGF-766
	126 CGTTGCTCTCCGACG	15	155 VEGF-r-1062
	127 GTAAAACTGGATCATCTC	16	156 U16720

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Claims

1. A pharmaceutical composition comprising at least one TGF-beta antagonist and at least one substance inhibiting cell proliferation and/or inducing cell death.
- 5 2. The pharmaceutical composition of claim 1 wherein the at least one TGF-beta antagonist and the at least one substance inhibiting cell proliferation and/or inducing cell death are mixed together.
3. The pharmaceutical composition of claim 1 wherein the at least one TGF-beta antagonist and the at least one substance inhibiting cell proliferation
10 and/or inducing cell death are separate.
4. The pharmaceutical composition according to claim 1 to 3 wherein the at least one TGF-beta antagonist is selected from the group of substances
 - inhibiting the production of TGF-beta and/or the TGF-beta receptor,
 - inhibiting TGF-beta and/or the TGF-receptor
 - 15 - inhibiting the function of TGF.
5. The pharmaceutical composition according to claim 1 to 4 wherein the at least one TGF-beta antagonist is selected from the group of
 - oligonucleotides hybridising with an area of the messenger RNA (mRNA) and/or DNA encoding TGF-beta,
 - 20 - TGF-beta receptors and/or parts of them binding TGF-beta,
 - antibodies and/or parts of them inhibiting TGF-beta,
 - proteins and/or peptides inhibiting TGF-beta,
 - molecules of less than 10,000 Da inhibiting the TGF-beta activity.
6. The pharmaceutical composition according to claim 5 wherein the oligonucleotide comprises at least one of the sequences with Seq. ID. No. 1-127
25 identified in the sequence listing.
7. The pharmaceutical composition according to claims 6 wherein at least one nucleotide of the oligonucleotide is modified at the sugar moiety, the base

and/or the internucleotide linkage.

8. The pharmaceutical composition according to claim 7 wherein at least one modified internucleotide linkage is a phosphorothioate linkage.

5 9. The pharmaceutical composition according to claim 1 to 8 wherein the at least one substance inhibiting cell proliferation and/or inducing cell death is selected from the group of antineoplastic chemotherapeutic agents.

10. The pharmaceutical composition according to claim 9 wherein the antineoplastic chemotherapeutic agent is selected from the group of ACNU, BCNU, CCNU, cisplatin, cyclophosphamide, liposomal pegylated doxorubicin, 5-
10 fluorodeoxyuridine, 5-fluorouracil, 5-fluorouridine, gemcitabine, procarbazine, taxol, taxotere, temozolomide, vinblastine, vincristine and their active derivatives.

11. The pharmaceutical composition according to claim 9 wherein the antineoplastic chemotherapeutic agent is temozolomide and/or its active derivatives.

15 12. The pharmaceutical composition comprising at least one stimulator of the function of the immune system and/or immune cells and at least one substance inhibiting the cell proliferation and/or inducing cell death.

13. The pharmaceutical composition of claim 12 wherein the at least one stimulator of the function of the immune system and/or immune cells at
20 least one substance inhibiting cell proliferation and/or inducing cell death are separate.

14. The pharmaceutical composition of claim 12 wherein the at least one stimulator of the function of the immune system and/or immune cells and the at least one substance inhibiting cell proliferation and/or inducing cell
25 death are mixed together.

15. A pharmaceutical composition according to one of the claims 12 to 14, wherein the at least one stimulator of the function of the immune system and/or the immune cells is

- stimulating and/or enhancing the synthesis and/or the function of cy-

tokines such as GM-CSF, SCF, CSF, IFN, FLT-3-ligand, monocyte chemotactic proteins (MCP-1), lymphotactin, interleukin-2, interleukin-4, interleukin 6, interleukin-12, interleukin-18 and/or interferone gamma or is one of these cytokines;

-
- 5 - selected from the group consisting of viruses, viral antigens, antigens expressed in tumor cells or pathogens, but not in normal cells, organ specific antigens expressed in affected organs which are not essential for the organism, fusion cells of dendritic cells and tumor cells and dendritic cells itself
- 10 - an antagonist of factors negatively influencing the function of the immune system
- /or a vaccine
- and
- the at least one inhibitor and inhibiting cell proliferation and/or inducing cell death is an antineoplastic chemotherapeutic agent.
- 15
16. The pharmaceutical composition according to claim 15 wherein the antagonist of factors negatively influencing the function of the immune system is selected from the group of DNA- or RNA fragments, antisense oligonucleotides, their active derivatives, antibodies, parts of antibodies, proteins, receptors, parts of receptors, peptides and molecules of less than 10,000 Da.
- 20
17. The pharmaceutical composition according to claim 16 wherein the antisense oligonucleotide is an oligonucleotide hybridising with an area of the messenger RNA (mRNA) and/or DNA encoding TGF-beta, VEGF, PGE₂ or IL-10 and/or their receptors.
- 25
18. Use of a composition comprising at least one TGF-beta antagonist and at least one substance inhibiting cell proliferation and/or inducing cell death for the preparation of a pharmaceutical composition for the treatment of neoplasms.
19. Use of a composition comprising at least one stimulator of the function of

the immune system and/or or immune cells and at least one substance inhibiting the cell proliferation and/or inducing cell death for the preparation of a pharmaceutical composition for the treatment of neoplasms.

20. Use of compositions according to claim 18 or 19 for the treatment of neo-

5 plasms selected from the group of bile duct carcinoma, bladder carcinoma, brain tumor, breast cancer, bronchogenic carcinoma, carcinoma of the kidney, cervical cancer, choriocarcinoma, cystadenocarcinome, embrional carcinoma, epithelial carcinoma, esophageal cancer, cervical carcinoma, colon carcinoma, colorectal carcinoma, endometrial cancer, gallbladder cancer,
10 gastric cancer, head cancer, liver carcinoma, lung carcinoma, medullary carcinoma, neck cancer, non-small-cell bronchogenic/lung carcinoma, ovarian cancer, pancreas carcinoma, papillary carcinoma, papillary adenocarcinoma, prostata cancer, small intestine carcinoma, prostate carcinoma, rectal cancer, renal cell carcinoma, skin cancer, small-cell bronchogenic/lung carcinoma, squamous cell carcinoma, sebaceous gland carcinoma,
15 testicular carcinoma, uterine cancer, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; pre-malignant tumors, astracytoma, blastoma, Ewing's tumor, craniopharyngloma, ependymoma, medulloblastoma, glioma, hemangloblastoma, Hodgkins-lymphoma, medullablastoma, leukaemia, mesothelioma, neuroblastoma, neurofibroma,
20 non-Hodgkins lymphoma, pinealoma, retinoblastoma, retinoblastoma, sarcoma (including angiosarcoma, chondrosarcoma, endothelialsarcoma, fibrosarcoma, leiomyosarcoma, liposarcoma, lymphangioendotheliosarcoma, lyphangiosarcoma, melanoma, meningioma, myosarcoma, oligodendroglioma, osteogenic sarcoma, osteosarcoma), seminoma, trachomas,
25 Wilm's tumor.

21. Use of one of the compostions according to claim 18 or 19 for the preparation of a pharmaceutical composition for the treatment of glioma and/or anaplastic astrocytom.

30 22. Method of treating neoplasms comprising the step of administering at least one TGF-beta antagonist in combination with

a) at least one substance inhibiting the cell proliferation and/or inducing cell death and/or

b) the step of applying radiation.

23. Method of treating neoplasms comprising the step of administering at least one stimulator of the function of the immune system and/or or immune cells in combination with

a) at least one substance inhibiting the cell proliferation and/or inducing cell death and/or

b) the step of applying radiation.

24. Method of treating neoplasms according to claim 22 or 23 wherein the neoplasm is selected from the group of: bil duct carcinoma, bladder carcinoma, brain tumor, breast cancer, bronchogenic carcinoma, carcinoma of the kidney, cervical cancer, choriocarcinoma, cystadenocarcinome, embryonal carcinoma, epithelial carcinoma, esophageal cancer, cervical carcinoma, colon carcinoma, colorectal carcinoma, endometrial cancer, gall-bladder cancer, gastric cancer, head cancer, liver carcinoma, lung carcinoma, medullary carcinoma, neck cancer, non-small-cell bronchogenic/lung carcinoma, ovarian cancer, pancreas carcinoma, papillary carcinoma, papillary adenocarcinoma, prostata cancer, small intestine carcinoma, prostate carcinoma, rectal cancer, renal cell carcinoma, skin cancer, small-cell bronchogenic/lung carcinoma, squamous cell carcinoma, sebaceous gland carcinoma, testicular carcinoma, uterine cancer, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; premalignant tumors; rheumatoid arthritis; psoriasis; astracytoma, acoustic neuroma, blastoma, Ewing's tumor, astracytoma, craniopharyngloma, ependymoma, medulloblastoma, glioma, hemangloblastoma, Hodgkins-lymphoma, medullablastoma, leukaemia, mesothelioma, neuroblastoma, neurofibroma, non-Hodgkins lymphoma, pinealoma, retinoblastoma, retinoblastoma, sarcoma (including angiosarcoma, chondrosarcoma, endothelial sarcoma, fibrosarcoma, leiomyosarcoma, liposarcoma, lymphangioan-

dotheliosarcoma, lymphangiosarcoma, melanoma, meningioma, myosarcoma, oligodendroglioma, osteogenic sarcoma, osteosarcoma), seminoma, trachomas, Wilm's tumor.

25. Methode of treating neoplasms according to claim 22 to 24 wherein the

5 step of administering an pharmaceutical composition is before or applying radiation.

26. Methode of treating neoplasms according to claim 22 or 24 wherein the step of administering a pharmaceutical composition is together with applying radiation.

10 27. Methode of treating neoplasms according to claim 25 or 26 wherein the total amount of radiation within one cycle is from about 10 Gy to about 100 Gy.

15 28. Methode of treating neoplasms according to claim 27 wherein the total amount of radiation of one cycle is applied by several fractions from of about 1 Gy to about 2 Gy.

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Abstract

The invention concerns a pharmaceutical composition comprising at least one stimulator of the immune cell functions and at least one substance inhibiting the cell proliferation and/or inducing cell death.

- 5 In a preferred embodiment the stimulator of the function of the immune system and/ or the immune cells are antagonists of TGF-beta.

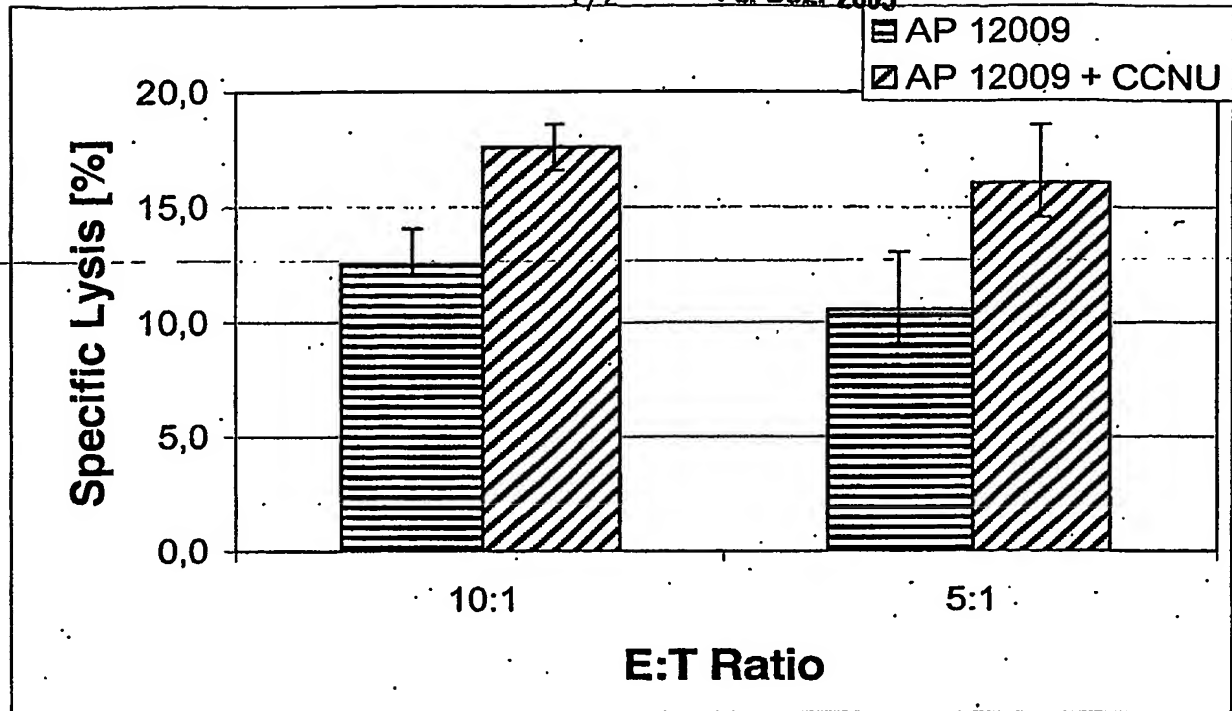


Figure 1

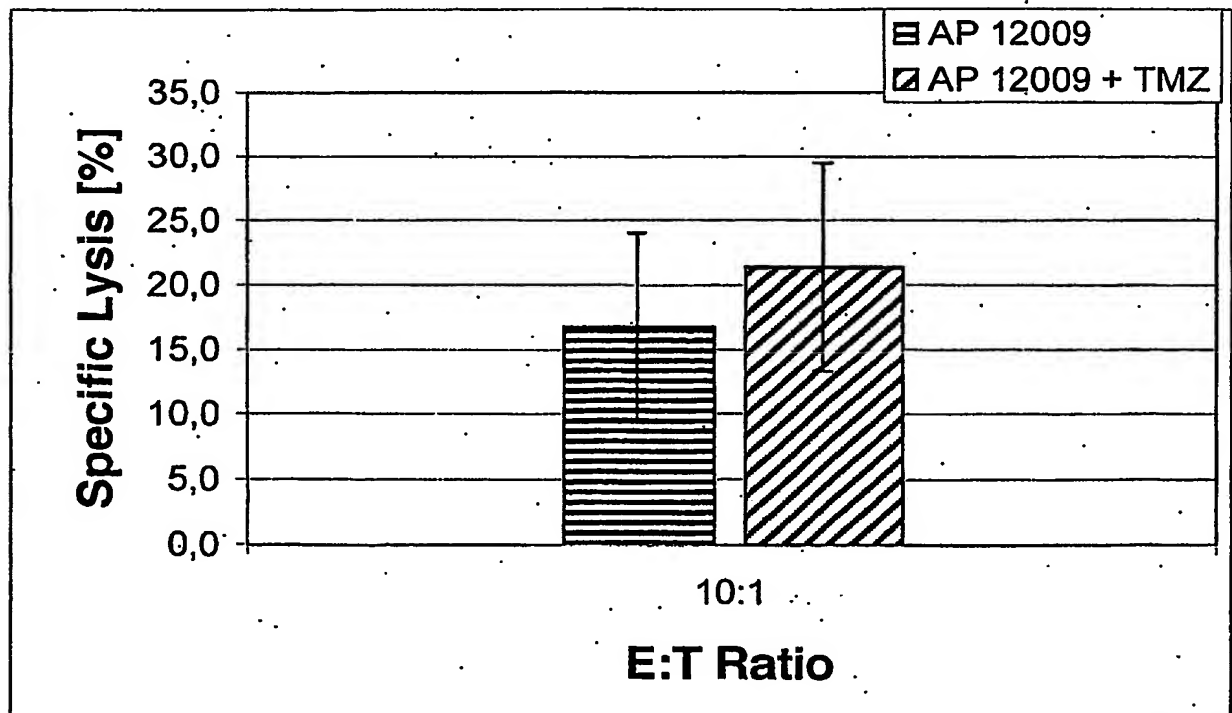


Figure 2:

Data as per 10/12/03

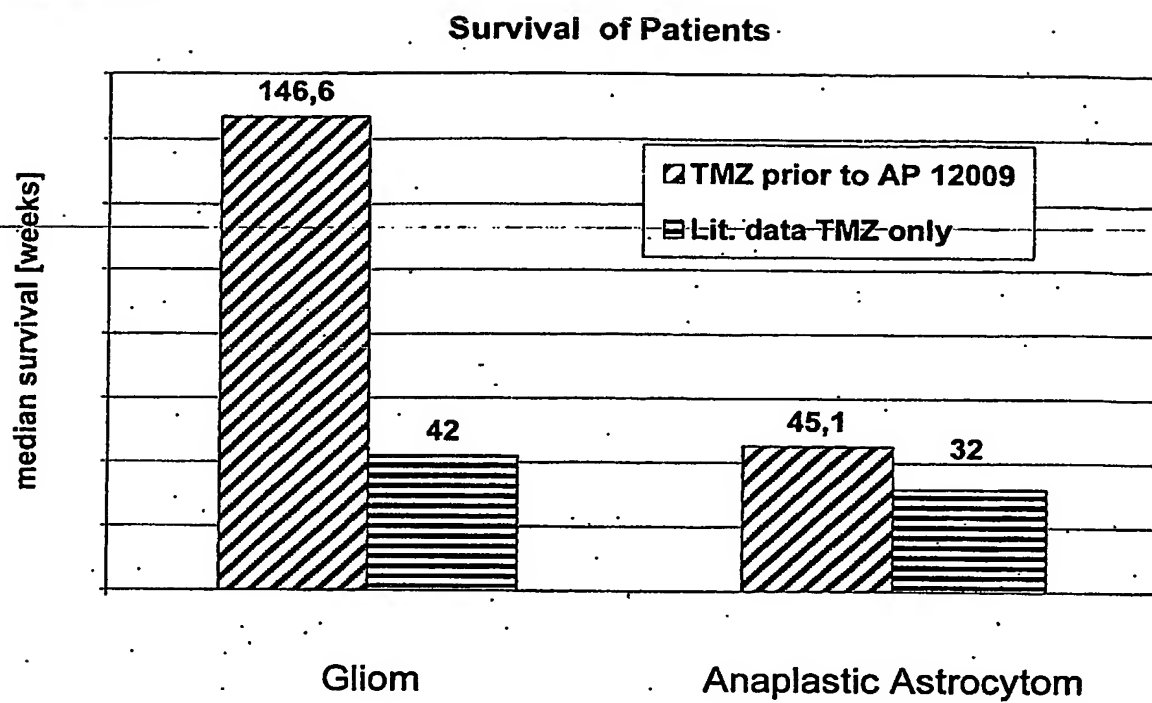


Figure 3

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